ISOLATION AND CHARACTERIZATION OF BACTERIAL PATHOGENS CAUSING ASYMPTOMATIC BACTERIURIA IN ANTENATAL WOMEN ATTENDING TERTIARY CARE HOSPITAL

Dissertation Submitted To

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

In partial fulfillment of the regulations

For the award of the degree of

M.D. (MICROBIOLOGY)

BRANCH IV

GOVT. KILPAUK MEDICAL COLLEGE

CHENNAI

April 2016

CERTIFICATE

This is to certify that this dissertation entitled **"ISOLATION AND CHARACTERIZATION OF BACTERIAL PATHOGENS CAUSING ASYMPTOMATIC BACTERIURIA IN ANTENATAL WOMEN ATTENDING TERTIARY CARE HOSPITAL "** is the bonafide original work done by **Dr. P. PRABHA,** Post graduate in Microbiology, under my overall supervision and guidance in the Department of Microbiology, Govt. Kilpauk Medical College, Chennai, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Microbiology (Branch IV).**

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DECLARATION

I solemnly declare that this dissertation "**ISOLATION AND CHARACTERIZATION OF BACTERIAL PATHOGENS CAUSING ASYMPTOMATIC BACTERIURIA IN ANTENATAL WOMEN ATTENDING TERTIARY CARE HOSPITAL"** is the bonafide work done by me at the Department of Microbiology, Govt. Kilpauk Medical College and Hospital, Chennai, under the guidance and supervision of **Dr.RADHIKA KATRAGADDA, M.D.,** Professor & H.O.D of Microbiology, **Dr. THYAGARAJAN RAVINDER, M.D.,** Professor, Department of Microbiology and **Dr. K.V. LEELA, M.D., DGO.** Associate Professor of Microbiology Department of Microbiology, Govt. Kilpauk Medical College, Chennai-600010. This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the University regulations for the award of Degree of M.D. Branch IV Microbiology examinations to be held in April 2016.

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ACKNOWLEDGEMENT

My heartfelt thanks and deepest sense of gratitude to **Dr.R.NARAYANA BABU, M.D., D.C.H.,** Dean**,** Government Kilpauk Medical College and Hospital for giving me permission to carry out my dissertation work and also to avail all the facilities available in the department.

I am deeply indebted to **Dr. RADHIKA KATRAGADDA, M.D.,** Professor and H.O.D., Department of Microbiology for her relentless efforts, valuable advice, excellent guidance and encouragement given to me throughout this study.

I am immensely grateful to **Dr. THYAGARAJAN RAVINDER, M.D.,** Professor, Department of Microbiology for his unflinching interest, effort and motivation extended to me during my study.

I owe my sincere gratitude to **Dr. K.V.LEELA, M.D.,** Associate Professor, Department of Microbiology for her valuable guidance, her timely advice, motivation and encouragement throughout the period of this study.

My sincere and special thanks to **Dr.A. Kala, M.D.,DGO** Professor and HOD, Department of Obstetrics & Gynaecology, Govt. Kilpauk Medical College and Hospital for her constant support and encouragement.

I extend my sincere thanks to **Dr.S.HEMALATHA,M.D., Dr. M. SUGANTHI, M.D.**, **Dr.K.LAVANYA,M.D., Dr.B.RAVICHANDRAN,M.D., Dr.C.AMUTHA,M.D.,** Assistant Professors, Department of Microbiology for their help, support, interest and valuable hints.

I also thank all my department Postgraduates for their timely help, cooperation and support. I express many thanks to all the technical staffs and other staff members of the Department of Microbiology for their kind co-operation to carry out this work successfully.

I also extend my thanks to all the patients who participated in my study.

I also thank my family for their endless love and moral support.

ABSTRACT

TITLE: ISOLATION AND CHARACTERIZATION OF BACTERIAL PATHOGENS CAUSING ASYMPTOMATIC BACTERIURIA IN ANTENATAL WOMEN ATTENDING TERTIARY CARE HOSPITAL

INTRODUCTION

Urinary tract infections (UTI) are one of the most prevalent bacterial infections and the significant cause of morbidity and mortality. Urinary tract infection is mainly a disease of the females, due to the anatomy of the female urethra. Escherichia coli are responsible for 50%-90% of infections. The gold standard screening test for asymptomatic bacteriuria is the urine culture. The management of asymptomatic bacteriuria in pregnancy promptly reduces the perinatal complications.

AIMS

1. To isolate and characterize the bacterial pathogens causing asymptomatic bacteriuria in antenatal women.
2. To determine the antibiotic sensitivity pattern of the isolates, ESBL and MRSA detection.

MATERIALS AND METHODS

1000 antenatal women were screened for asymptomatic bacteriuria at Government Kilpauk Medical College and Hospital. Urine specimens were collected by mid-stream clean catch method and processed immediately. To isolate the organism, a semi-quantitative calibrated loop technique was used. The virulence

factors of the organisms were determined by virulence tests. Antibiotic sensitivity testing was performed by the Kirby-Bauer's disc diffusion method as per CLSI guidelines 2014(M100-S24). Detection of ESBL's were done by phenotypic confirmatory test, MIC and ESBL gene by PCR.

RESULTS

The prevalence of asymptomatic bacteriuria in pregnant women was 11.8%, more in the 21-30 years, primigravida and first trimester. Escherichia coli was the common isolate 54(45.76%) with Haemolysin18(33.33%) as the major virulence factor. MRSA was not detected. Enterobacteriaceae showed 100% sensitivity to imipenem and piperacillin/tazobactam. Sensitivity to Cefotaxime and ceftazidime was 92.6% for Escherichia coli, 89.5% for Klebsiella pneumoniae.

Among Enterobactericeae, 6 were ESBL producers by phenotypic confirmatory test. Out of 6, 3(50%) were positive for CTX-M gene. 9 Escherichia coli isolates were positive for Mannose resistant haemagglutination and pap A gene.

CONCLUSION

Asymptomatic bacteriuria in pregnant women which if not treated, might lead to various perinatal complications. Therefore, routine urine culture should be done for all antenatal women, to detect asymptomatic bacteriuria, and every positive case should be treated.

Keywords: Asymptomatic bacteriuria, Pregnancy, Escherichia coli, Virulence factors, ESBL.

INTRODUCTION

Urinary tract infections (UTI) are one of the most prevalent bacterial infections and the significant cause of morbidity and mortality[1,2,3]. Urinary tract infection is next to respiratory tract infection, particularly in females[4]. Urinary tract infection is mainly a disease of the females, due to the anatomical structure of the female urethra[1].

Of all uncomplicated urinary tract infections, Escherichia coli are responsible for 50%-90% of infections. They are mainly from faecal flora that can colonise the periurethral region, defeat the local host defences, enters and grow within the urinary tract[1]. The other bacterial pathogens that cause Urinary Tract infection are Klebsiella pneumoniae, Proteus species, Enterobacter species, Staphylococcus saprophyticus and Streptococcus agalactiae[5]. In pregnancy, urinary tract infections commonly occur, because of the physiological and morphological changes that occur in the genitourinary tract[6].

There are 2 types of urinary tract infection (UTI). They are asymptomatic and symptomatic urinary tract infection[6, 7, 8]. The definition of asymptomatic bacteriuria is the occurrence of actively multiplying bacteria, more than 10^5 bacteria per ml of urine inside the urinary tract, exclusive of the distal urethra at a time when the patient has nil symptoms of UTI[9, 10]. Asymptomatic bacteriuria is frequent in women and more in occurrence with age, sexual activity. This is because of the short urethra, antenatal period and faecal contamination of the urinary tract easily[11, 12].

Antenatal women are twice more frequently affected than the same aged non-pregnant women due to urinary stasis which is because of the progesterone effect in antenatal period in addition to various physiological and structural changes that occur in pregnancy[10]. In pregnant women, the occurence of asymptomatic bacteriuria was found to be 2% to 10%[11, 12].

Pregnancy increases the succession from asymptomatic bacteriuria to symptomatic bacteriuria which can cause acute pyelonephritis in 20 to 50% of cases and contributes to adverse perinatal outcomes like post partum hypertensive disease, urinary tract infections, anaemia, prematurity and increased fetal mortality rates if left untreated[6,13,14].

Asymptomatic bacteriuria is a microbial diagnosis and depends on the isolation of bacteria which is specific and quantitatively significant in a specimen of urine that is collected properly from pregnant women without any signs and symptoms of UTI. Hence detection and early treatment decreases the incidence of obstetric complications like acute pyelonephritis and chronic kidney failure not only in mother but it also reduces the premature delivery and fetal death[6]. The gold standard screening test for asymptomatic bacteriuria is the urine culture[6, 11, 15].

The relatively increased occurence of asymptomatic bacteriuria, the consequences faced by the antenatal women, the ability to avoid undesirable outcomes with the management justifies the testing of asymptomatic bacteriuria in pregnancy[6].

In different geographical regions, the frequency of the pathogen isolated and their antimicrobial resistance patterns can vary. Hence, the common etiological

agents of asymptomatic bacteriuria should be investigated and their antimicrobial resistance pattern to be made aware of [6]. The strains of Escherichia coli causing urinary tract infection are not like the faecal isolates[1].

The virulence factors like adhesions, toxins, gelatinase enzyme and the surface hydrophobicity have an influence on the pathogenicity of the organism. The occurence, expression of the virulence genes, the environmental conditions in the host determine the virulence of the individual strains in a given infection. The colonisation of the organisms and the tissue damage is because of these virulence factors[1].

Uropathogenic Escherichia coli (UPEC) strains are mostly marked by the presence of certain virulence determinants. Uropathogenic Escherichia coli have chromosomal gene clusters on pathogenicity islands and they encode adhesins such as S pili, P pili and afimbrial adhesins and other virulence genes[16].

Antenatal women with asymptomatic bacteriuria are prone to develop postpartum urinary tract infection, acute pyelonephritis in later half of pregnancy, anemia, chronic kidney failure, babies of low birth weight, prematurity and perinatal death if not treated. The management of asymptomatic bacteriuria in pregnancy promptly reduces the occurence of all these complications[17].

AIMS & OBJECTIVES

AIMS AND OBJECTIVES

1. To isolate the bacterial pathogens causing asymptomatic bacteriuria in antenatal women.
2. To find the virulence factors of the bacterial isolates.
3. To determine the antibiotic sensitivity pattern of the isolates.
4. To detect the Extended Spectrum Beta Lactamase (ESBL) producers from Gram negative bacterial isolates.
5. To find the prevalence of Methicillin Resistant Staphylococcus aureus (MRSA).

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Bacteriuria is a commonly used term and literally means "Bacteria in the urine". Infections in the urinary tract are one of the most frequent of all infections. The presence of infected urine in the bladder depends on the quantification of the number of bacteria in the excreted urine. Significant bacteriuria is a term used to describe the number of bacteria in voided urine that is (10^5 bacteria/ml). The presence of atleast 10^5 bacteria/ml of urine, infection must be considered as a serious one[18].

Kass in 1957 suggested that 100000 bacteria/ml of urine is significant bacteriuria. In real infections, in patients who have not taken any antibiotics previously, the number of bacteria is $\geq 10^5$/ ml of urine. So, a quantitative method is adopted for the culture of viable bacteria in the specimen[19].

Among the clinical presentations of urinary tract infection, asymptomatic bacteriuria is also present. The definition for asymptomatic bacteriuria is actively, persistently growing bacteria in significant counts (10^5 bacteria per ml of urine) without any obvious symptoms. Covert bacteriuria is the other name of it. The short urethra in females contributes to this. The antenatal women are twice more commonly affected than same aged non-pregnant women. The progesterone effect in pregnancy leads to urinary stasis is the reason behind this increase in pregnant women. The anatomical changes that occur also contribute to it[10].

In women, asymptomatic bacteriuria is frequent and it increases with age and sexual activity. The reasons are short urethra, absence of prostatic secretions and easy contamination of urinary tract with faecal flora in pregnancy[11].

In the antenatal period, various changes occur in the body of women. Mechanical and hormonal changes enhance the problem of stasis in the bladder and reflux from the bladder to the ureters. The changes are short urethra, problem in maintaining the hygiene because of the distended abdomen. All these enhance the occurence of urinary tract infections. The smooth muscle relaxations followed by ureteral dilatation in antenatal period facilitate the bacteria to move from the bladder to the kidneys[9].

Asymptomatic bacteriuria occurs in 2-10% of antenatal women. The detection of asymptomatic bacteriuria in pregnancy is significant, as untreated asymptomatic bacteriuria can cause symptomatic infection in 25% of culture positive cases, post partum urinary tract infection, pregnancy induced hypertension, anaemia, acute pyelonephritis, preterm birth, low birth weight babies and death of the foetus[11].

The gold standard technique for the screening of asymptomatic bacteriuria in antenatal period is the culture of urine. Escherichia coli is the most common infecting organism and accounts for 75-90% of bacteriuria in pregnancy. 25-30% of asymptomatic bacteriuria women progress to symptomatic urinary tract infection. Thus, early detection and treatment is important to prevent the complications both in mother and the baby[11].

PREGNANCY INDUCED URINARY TRACT CHANGES[20,21]:

In pregnancy, significant changes occur in the structure and function of the urinary tract. The kidneys become larger, renal calyces and ureters dilate. Some dilatation occurs before 14 weeks and is because of the progesterone induced relaxation of the muscular layers. In mid-pregnancy, marked dilatation is obvious. This is because of ureteral compression, mainly on the right side. During pregnancy vesicoureteric reflux is also there. An enhanced risk of upper urinary infection is due to the consequence of these physiological changes.

Soon after conception functional hypertrophy becomes evident. The size of the glomeruli increases, whereas the number does not increase. Increase in the effective renal plasma flow and glomerular filtration is due to the pregnancy induced intra renal vasodilatation. The glomerular filtration rate is increased by 20%, than the non pregnant values by 12 weeks of gestation. The plasma flow increases by 40% and glomerular filtration increases by 65% thereby the concentrations of creatinine and urea decreases. Other changes include those involved in the maintainence of normal acid-base homeostasis, osmoregulation, and fluid and electrolyte retention. The increase in plasma volume occurs physiologically in antenatal period. This decreases the concentration of urine. Most antenatal women get glucosurea, which promotes the growth of bacteria in the urine.

THE NORMAL FLORA OF THE URINARY TRACT[22,23]

- Coagulase negative Staphylococci (excluding S.saprophyticus)
- Non hemolytic and Viridans Streptococci
- Lactobacilli
- Non pathogenic Neisseria species
- Diphtheroids (Corynebacterium species)
- Anaerobic cocci
- Anaerobic Gram negative bacilli
- Propionibacterium species
- Commensal Mycobacterium species
- Commensal Mycoplasma species

HISTORICAL BACKGROUND

In 1957, Kass proposed the use of quantitative culture of urine for the testing of urinary tract infection. Asymptomatic patients with counts quantitative of $\geq 10^5$ (CFU)/ml of excreted urine had the similar organisms when isolated in paired specimens using urinary catheters[19].

Several patient populations, who were asymptomatic clinically, had a positive urine culture. These included pregnant women, cases with urinary catheters insitu and individuals with urological abnormalities. Pyelonephritis was identified as a vital difficulty in antenatal women with asymptomatic bacteriuria[24].

EPIDEMIOLOGY

As per the Cochrane collaboration study, 2-10% of all pregnancies, contribute to asymptomatic bacteriuria[25]. In a study done at Chennai by Balamurugan et al the prevalence is found to be 13%[3]. In most of the developing countries, recent studies show same statistics. In a cohort study of 9734 antenatal women, 5.1% had asymptomatic bacteriuria, 7.4% were found to have a urinary tract infection followed by 1.3% acute cystitis and 1% acute pyelonephritis respectively. The occurence of pyelonephritis in pregnant women ranges from 0.5 to 2%. Second half of pregnancy is more risky for pyelonephritis. This is because of the rising mechanical compression of the distended uterus.

Socioeconomic status is important in determining the prevalence of asymptomatic bacteriuria. Associated risk factors are history of diabetes, urinary tract infections occurring recurrently and anatomical anomalies of the urinary tract. The effects of host factors like age, parity, race, sickle cell disease are all less clear.

Asymptomatic bacteriuria plays a major role for symptomatic UTI. Asymptomatic bacteriuria if not treated 30% of pregnant women develop pyelonephritis when compared to 1.8% of non-bacteriuric controls[26].

ETIOLOGIC AGENTS OF ASYMPTOMATIC BACTERIURIA[26, 27, 28]

The aetiologic agents of bacteriuria are the same in non-pregnant and pregnant women. Major commonest pathogen is Escherichia coli contributing to 70-80%[26]. Escherichia coli strains isolated from asymptomatic bacteriuria women have

fewer virulence characteristics than those of symptomatic women. Other organisms like Klebsiella pneumoniae, Proteus mirabilis, coagulase-negative staphylococci, group B streptococci and Gardenella vaginalis are common as well[27]. Other organisms causing asymptomatic bacteriuria are Enterobacter species and Pseudomonas aeruginosa[28].

Streptococcus agalactiae in urine has been associated with labour and neonatal complications. Significance of the presence of anaerobic and fastidious organisms in the urine of antenatal women is unknown[26].

PATHOPHYSIOLOGY

The occurence of bacteriuria without symptoms in non pregnant and antenatal women are the same, but it is usually benign in nonpregnant women. Outflow obstruction of urine in pregnant women causes stasis and in turn leads to pyelonephritis which complicates the asymptomatic bacteriuria. Physiological changes of the urinary tract that occur in pregnancy promote the development of asymptomatic bacteriuria to pyelonephritis. Progesterone induced dilatation of the ureters and renal pelvis, dislodgement of the bladder into the abdomen and stasis of urine due to reduced ureteral and bladder tone is the reason behind[29]. In foetus, the effects of asymptomatic bacteriuria are premature labour, babies with low birth weight and death of the foetus[11].

Virulence factors in uropathogenic strains of Escherichia coli include toxins, fimbriae and adhesins that allow attachment to uroepithelial cells that inhibit bacteria from urinary lavage, allows tissue invasion, multiplication, invasive infection

and pyelonephritis in antenatal women subsequently. Adherence is the single most important marker associated with the progression to pyelonephritis[29].

The virulence factors of Staphylococcus aureus are capsular polysaccharides, peptidoglycan, teichoic acid, protein A, enzymes, hemolysins, toxins and super antigens. The virulence factors of Pseudomonas aeruginosa are alginate, pili, neuraminidase, lipopolysaccharide, exotoxin A, enterotoxin, exoenzyme S, phospholipase C, elastase, leukocidin and pyocyanins[30].

PATHOGENESIS

Bacteria that originate in the large bowel most likely colonise the urinary tract transperineally. Escherichia coli cause 75-90% of bacteriuria in antenatal women. The pathogenic virulence of Escherichia coli which is not the most plentiful in faecal isolates derives a number of factors like resistance to vaginal acidity, adherence to cells, rapid division in urine and the production of chemicals which reduce ureteric peristalsis and inhibit phagocytosis. Other organisms responsible for urinary tract infection include Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas and coagulase-negative staphylococci. The interaction between infection and the host is an interesting area of research and much is discovered about the roles of bacterial adhesions, uro-epithelial receptors and induction of inflammatory responses[22].

Adherence of the organisms to epithelial cells is important for the colonization and infection of mucous membranes. The colonization of the urogenital epithelium with the bacteria of susceptible persons is related to the successful invasion of the urinary tract. Urine and serum antibodies play an important part in the

pathogenesis of UTI. The adherence of bacteria to the uroepithelial cells is prevented by the urinary antibodies and thereby they resist UTI[31].

Mostly the organisms enter into the bladder, via the urethra. An insitu catheter helps their invasion which colonizes in the periurethral area, vaginal region and ascends. Bacteria that cause infection in healthy women and in persons with complications express different virulence properties[32]. The low sensitivity of the bacteria to the anti microbial agents used is the major problem associated with infections caused by biofilm forming bacteria[33].

VIRULENCE FACTORS OF ORGANISMS

Adhesins

Increased adherence to periurethral and vaginal area promotes colonization. Pyelonephritis causing isolates are virulent than cystitis causing isolates. Urinary isolates are more virulent than colonic isolates. The adhesins of uropathogenic Escherichia coli occur as filamentous surface organelles termed fimbriae or as nonfilamentous proteins of outer membrane[18].

P Fimbriae

The adherence of Escherichia coli to the epithelial cell receptors having globoseries glycosphingolipid is related to the attachment of most strains causing renal infection and is not inhibited by mannose - Mannose-resistant (MR). P fimbriae are a part of the P blood group antigen complex found in human red blood cells. The globoseries glycosphingolipid receptors (gal-gal) are present throughout the urinary tract, mainly in the kidney[18].

Type I Fimbriae[18, 32]

Mannose-sensitive hemagglutination is due to type I fimbriae found in many enterobacterial species and especially in many strains of Escherichia coli, commonly expressed by isolates from cystitis than by control isolates and pyelonephritis isolates. Pil, fim gene cluster and other nine genes code for the type I fimbriae, proteins of structure and regulation. Type I fimbriae undergo phase variation which is because of an invertible promoter. Liquid media promotes the production of fimbriae. Bladder colonizers are generally fimbriated than kidney isolates. Type I fimbriae gets attached to uroplakins, the major glycoprotein of the uroepithelial cells. Type I fimbriae provoke invasion of the urinary bladder epithelial cells and recurrent infections.The bacteria within the urothelial cell form biofilm within a polysaccharide.

Other adhesins[18]:

In addition to type P and type I fimbriae, various other adhesins include S, G type 1 c, Dr fimbriae, and X and M adhesins with differing molecular binding specificities and serologic properties have been found on uropathogenic strains of Escherichia coli. The Dr hemagglutinin family has non fimbrial and fimbrial adhesins. Four genes (dra A, B, C, D) that encode for the structural proteins and adhesins of the fimbriae have been found. The adhesins attach to the Dr blood group antigen component of decay accelerating factor, which is widely present along the urinary

tract and cause cellular invasion. Dr positive Escherichia coli persist in renal infections and have a role in interstitial nephritis and chronic pyelonephritis.

Adherent bacteria persist in the urinary tract and have growth advantages and increased toxicity. Dr adhesin and the Type I pili have been linked together to bladder epithelial cell invasion and intracellular persistence by uropathogenic Escherichia coli. A positive correlation is there between the bacterial adhesion and the difficulty in eradicating organisms with short course antibiotic therapy.

Studies with other bacterial uropathogens like Klebsiella species and Proteus mirabilis have also demonstrated the importance of adherence in the pathogenesis of UTI. Staphylococcus aureus rarely causes cystitis and ascending pyelonephritis, whereas, Staphylococcus saprophyticus is a frequent reason for lower urinary tract infections. Staphylococcus saprophyticus adheres more significantly to uroepithelial cells than Staphylococcus epidermidis or Staphylococcus aureus isolates[18].

OTHER VIRULENCE FACTORS:

Motile bacteria go up in the ureter against the flow of urine. The endotoxins of Gram negative bacilli reduce the peristalsis in the ureter and add to the renal parenchymal inflammation by phagocytic cell activation. In Proteus species, the urease produced by the infective microorganisms has the ability to cause pyelonephritis. The capsular K antigen present in the bacteria protects it from phagocytosis.

The hemolysin produced by most uropathogenic strains cause tissue invasion and renal tubular epithelial and parenchymal damage, making iron available to the invading Escherichia coli. The hemolysin gene is mostly located adjacent to genes encoding for serum resistance and sialic acid - specific (S) fimbriae[18]. Hemolysin provides Escherichia coli with selective advantage by releasing iron from lysed erythrocytes and increases the pathogenicity by destroying epithelial and phagocytic cells[34].

In uropathogenic strains of Escherichia coli, aerobactin an iron scavenging protein (siderophore) is present in higher frequency. Because of the conditions in the bladder are iron poor, iron acquisitions systems are often found in uropathogenic Escherichia coli. Increased number of iron acquisition systems is present in uropathogenic Escherichia coli strains than the commensal and fecal strains, which reflects the iron limiting urinary tract environment[18].

Biofilms consists of mass of bacteria attached to a surface and covered within an extracellular matrix. Changes in gene and protein expression, as well as metabolic activity contribute resistance to host mechanisms of clearance and antimicrobial therapy in biofilms[35].

Virulence genes

Genes that have been identified that encode for the virulence factors are hlya (hemolysin), cnf1 (cytotoxic necrotizing factor1), iutA (aerobactin receptor), fim H (Type 1 fimbriae adhesion), pap (p fimbriae), iroN (catecholate siderophore receptor), omp T (outer membrane protease T). Cytotoxic necrotizing factor 1(cnf) causes

urinary tract infection and prostatitis. Iron regulated gene homologue adhesions (Iha), an outer membrane protein encoded by iha are virulence factor for urinary tract infection. Escherichia coli strains causing asymptomatic bacteriuria appear to have lost their ability to express functional virulence associated genes[18, 36].

HOST DEFENCE OF THE URINARY TRACT[18]:

1. Urine (osmolality, Ph, organic acids)
2. Mucosa of the urinary tract (Bactericidal, peptides, cytokines)
3. Urinary inhibitors to prevent bacterial adherence
4. Urine flow and micturition
    - Tamm-Horsfall protein
    - Mucopolysaccharide of the bladder oligosaccharides
    - Secretory immunoglobulin A
    - Lactoferrin.
5. Inflammation

    Polymorphonuclear neutrophils, cytokines.

6. Immunity

    Humoral and cell mediated immunity

7. Others

    Prostatic secretions

LAB DIAGNOSIS

Sample collection[30]

Clean catchment of midstream urine is the most commonly used technique to collect the urine sample to avoid the contamination. The initial flow washes away the contaminants whereas the midstream urine reflects the bacterial colonization of the bladder. Periurethral cleansing with water and soap without bactericidal activity is insisted to reduce the contamination in females as culture results are modified with antibacterial soap.

Midstream clean catch urine is the most commonly used technique for sample collection. The periurethral area and the perineum should be first cleaned with two or three gauze pads, soaked in soapy water, using a back to forward motion. Then the periurethral area and perineum should be rinsed with water or sterile saline.

The labia should be held apart during voiding, and the first few millilitres of urine should be passed into a bedpan or toilet to flush out the bacteria from the urethra. The midstream portion of urine should then be collected in a wide mouthed sterile container and covered with a tightly fitted lid[30].

Specimen transport

Urine sample is processed within 2-4 hours or should be refrigerated to maintain the bacterial density. Preservative advised is 1.8% Boric acid where specimen to be transported and refrigeration is not possible. Results after prolonged storage will alter the culture results[32].

Examination of urine

Macroscopic examination

Urine to be observed for altered colour, presence of turbidity and deposits[37].

Screening tests for presumptive diagnosis of significant bacteriuria

Leukocyte esterase test and nitrite test[17]

The presence of nitrite and leukocyte esterase was tested with colorimetric Combur-10 multireagent strips as per the manufacturer's instructions.

Pus cell count of the uncentrifuged urine[17]

With Neubauer's counting chamber pyuria was detected. Pus cell count of ≥10 cells/µl of urine corresponds to an excretion rate of $4* 10^5$ pus cells in 1 hour which is significant.

Gram staining of uncentrifuged urine[17]

1 drop of urine that was not centrifuged was taken on a clean slide and Gram staining done and examined for 20 fields. Presence of more than or equal to one bacteria per examined field indicates $\geq 10^5$ CFU/ml of urine.

Triphenyl Tetrazolium Chloride (TTC) test[4, 38]:

Only at an alkaline p^H reduction of Triphenyl tetrazolium chloride to a red precipitate of Triphenyl formazan occurs and this occurs during a urinary tract infection.

Catalase test[4, 38]

To a 2 ml sample of urine a few drops of hydrogen peroxide was added. Presence of the catalase enzyme was evidenced by frothing. A positive result is also obtained in hematuria.

Griess nitrite test[39]

The reagent consists of an acid solution of sulfanilic acid and alpha naphthylamine which undergoes diazotization with nitrites to form a red dye. Nearly all the bacteria which cause asymptomatic bacteriuria reduce the nitrate to nitrite.

Glucose test paper[38]

The bacteria causing the infection utilises the minute amounts of glucose present in normal urine.

Dip slide culture method[19, 38]

The dip-slide is a small plastic tray carrying a layer of an appropriate agar culture medium. Agar coated slides are immersed in urine or even exposed to the stream of urine during voiding incubated at 37 degree celcius overnight and the growth estimated by colony counting or by colour change of indicators.

Advantage

It is especially convenient for routine screening of large number of patients.

Disadvantage

It does not provide material for microscopical examination for the cellular content of the urine and when the bacterial count is high and when the growth of the dip-slide is confluent, it is difficult to judge whether the growth is pure or mixed.

IMPORTANCE OF SCREENING

For asymptomatic bacteriuria screening, no clear cut idea in the literature is present as to the timing and frequency of screening. In a study involving 3254 antenatal women in Sweden was examined for the risk of getting bacteriuria in pregnancy. The risk of getting the bacteriuria in pregnancy augmented from 0.8% in the I trimester to 1.93% at the III trimester. The risk of getting the infection was the most between the 9^{th} and 17^{th} week. 16^{th} gestational week was the most favourable time for screening. Management at that time will provide the maximum benefit. As per many studies, the urine culture done once at first trimester is adequate, whereas few studies report that it must be done in all the three trimesters to enhance the sensitivity[26].

Screening and treating the asymptomatic bacteriuria in antenatal women is important in obstetric care. Most of the pregnancy related guidelines add screening as a test for asymptomatic bacteriuria. Pyelonephritis has been prevented by the screening and management of asymptomatic bacteriuria and shown to be cost effective[25].

Urine culture[40]

A semi quantitative calibrated loop technique was used for the isolation of the organism. A loopful of urine that was uncentrifuged was inoculated on to the CLED and blood agar plates. The plates were incubated at 37^0C for 18-24 hours aerobically. The colony forming units per ml of urine was calculated. By standard microbiological techniques, bacterial isolates were identified and then antibiotic sensitivity testing was done by Kirby Bauer's disc diffusion method.

COMPLICATIONS[41]

Pregnant women with asymptomatic bacteriuria are prone to the risk of developing symptomatic UTI and acute pyelonephritis. Hill et al has reported an incidence of 1.4% of acute pyelonephritis in pregnant women. Smaill et al in a systematic review has shown the occurence of pyelonephritis in the untreated asymptomatic bacteriuria group to be 21% with a range of 2.5 to 36%. Management of asymptomatic bacteriuria leads to a 75% fall in the incidence of pyelonephritis. Proper management of asymptomatic bacteriuria also decreases the symptomatic UTI by 80-90%. The incidence of anemia was high in all the age groups despite treatment. The association between anaemia and asymptomatic bacteriuria could not be established because of the multipronged aetiopathogenesis of anemia in pregnancy. Asymptomatic bacteriuria in pregnancy is associated with IUGR, hypertensive disorders of pregnancy, Pre term labour, and low birth weight infants. All these complications emphasize the importance of early detection and management of asymptomatic bacteriuria.

TREATMENT

Since no proper idea is found in the studies regarding the preference of antibiotic and its duration of therapy, more than the clinical trials the treatment depends on the national patterns of antibiotic practice and its resistance. The antibiotic of choice should have high efficacy, decreased resistance and good fetal and maternal safety[26]. Management of asymptomatic bacteriuria has been shown to prevent pyelonephritis, management with a suitable antimicrobial agent is recommended for all pregnant women with significant bacteriuria. The aim of the treatment is to maintain sterile urine throughout pregnancy and thereby prevent the complications[18].

Treatment with a relatively non-toxic drug for 7 days eliminates bacteriuria in 70-80% of antenatal women. The relatively safe drugs in pregnancy are a sulphonamide, amoxicillin, cephalexin, amoxicillin-clavulanate and nitrofurantoin. Quinolones and tetracyclines must be avoided in pregnancy. Sulphonamides should not be given in the last few weeks of pregnancy because they cause hyperbilirubinemia and kernicterus in the newborn baby[18]. Nitrofurantoin has a good sensitivity (90.98%), against both ESBL and non ESBL producers. The absorption of nitrofurantoin when taken orally is 40-50% and so, it is taken with food. The drug has few adverse effects. It can be used safely for the management of uncomplicated cystitis in pregnancy[42, 43].

Urine cultures should be done 1 to 2 weeks after stopping the therapy and at regular intervals like monthly for the remaining gestational weeks. If bacteriuria recurs, treatment should be given for reinfection or relapse. Using catheters should be avoided during delivery[18].

Antibiotics commonly used for asymptomatic lower urinary tract infections[26]

DRUG	ADVANTAGES	DISADVANTAGES
Cephalexin	No tetatogenicity.	Penicillins and cephalosporins are associated with allergic manifestations occasionally.
Nitrofurantoin	Appears safe in all trimesters of pregnancy.	Not useful in pyelonephritis because therapeutic levels are achieved only in the urine. Not beneficial against Proteus species.
Trimethoprim-sulfamethoxazole	--	In first trimester, can cause neural tube and birth defects. Sulphonamides are avoided in III trimester to prevent neonatal toxicity.

BACTERIAL RESISTANCE[44]:

Resistance of microorganisms to drugs involve many mechanisms which are as follows

1. Certain microorganisms produce enzymes which destroy active drug. For ex. β lactamases produced by Staphylococci and Gram negative organisms.
2. Some of them change their permeability to the drug.
3. Developing an altered target for the drug or changed metabolic pathway which bypasses the reaction repressed by the drug.
4. Production of an altered enzyme that can perform its metabolic activity but very little action is altered by the drug.

METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS[30]

Staphylococcus aureus is an important human pathogen present in the surroundings and in the anterior nostrils of 20- 40% of adults. It is also seen in the axillae; intertriginous skin folds, the perineum, and the vagina. It is responsible for mild to severe life threatening infections including skin and soft tissue infections. Originally, penicillin was the treatment of choice for the management of serious Staphylococcus aureus infections. The advent of penicillin resistance in the Staphylococcus aureus was due to the acquisition of plasmid borne genetic elements coding for β lactamase production.

MECHANISM OF RESISTANCE

The chromosomally localized mecA gene is responsible for methicillin resistance. Penicillin binding protein 2a synthesis requires mecA gene. Penicillin binding protein is membrane bound enzyme which catalyses the transpeptidation effect which is responsible for cross linking of peptidoglycan chains. All beta lactam antibiotics have low affinity for PBP2a. It substitutes other PBPs which enables Staphylococcus to survive exposure to high concentration of beta lactam antibiotics[30].

Four different SCCmec elements have been recognized. SCCmec type I, II and III is associated with Health care associated MRSA (HA-MRSA). Community associated MRSA tend to carry Type IV element. CA - MRSA has a characteristic antibiotic susceptibility pattern, carries specific virulence factor such as Panton – valentine leukocidin and specific SCC mec type IV[45, 46].

BETA LACTAMASES

BETA LACTAM ANTIBIOTICS

Beta lactam antibiotics are the ones which contain β lactam ring in its structure. It includes Penicillins, Cephalosporins, Monobactams and Carbapenem[30].

MECHANISM OF ACTION[47, 48]

All beta lactam antibiotics inhibit the bacterial cell wall synthesis. The bacteria produce UDP - N – acetylmuramic acid, UDP – N – acetyl glucosamine. The peptidoglycan synthesis residues are linked together forming lengthy strands and the UDP is cut off. Terminal D alanine of the peptide chain is cleaved by the transpeptidases. The cross linking is necessary for the strength and rigidity of the cell wall. The beta lactam antibiotics inhibit the transpeptidases so that cross linking does not take place.

BETALACTAMASES

This is a heterogeneous group of penicillin recognizing proteins. They belong to members of super family of active site serine protease. These enzymes inactivate β lactam antibiotics[30].

CLASSIFICATION OF BETA LACTAMASES[49]

Schemes of functional classification that were accepted by β-lactamase researchers include:

(i) In 1968, Cephalosporinases and penicillinases were grouped on the basis of reaction to specific antibody (Sawai et al[50]).

(ii) In 1973, the Richmond and Sykes scheme classified the enzymes into five main divisions based on the gene coding for β-lactamase and the substrate profile.

(iii) In 1989, Bush scheme classified β-lactamase on the basis of molecular structure and the substrate inhibition.

(iv) In 1980, Ambler was the first to propose the Molecular structure classifications.

(v) More recently, Bush, Jacoby, and Medeiros devised a classification scheme based on the sequence of nucleotide on the genes for placing β-lactamases into functional groups and on the enzyme's biochemical properties and molecular structure.

CLASSIFICATION OF BETA LACTAMASES[49]

(Classification schemes for bacterial β-lactamases)

Bush-Jacoby-Medeiros group	1989 Bush group	Richmond-Sykes class	Mitsuhashi-Inoue type	Molecular class	Preferred substrates	Inhibited by:		Representative enzyme
						CA[b]	EDTA	
1	1	Ia,Ib,Id	Csase[a]	C	Cephalosporins	-	-	AmpC from Gram negative bacteria, MIR-1
2a	2a	not included	Pcase V	A	Penicillin	+	-	Penicillinases from Gram Positive bacteria
2b	2b	III	Pcase I	A	Penicillins, Cephalosporins	+	-	TEM-1, TEM-2, SHV-1
2be	2b'	not included except K1 in class IV	Cxase	A	Penicillins, Narrow spectrum and extended spectrum Cephalosporins, Monobactams.	+	-	TEM-3 to TEM-26, SHV-2 to SHV-6, *Klebsiella oxytoca* K1
2br	not included	not included	not included	A	Penicillins	±	-	TEM-30 to TEM-36, TRC-1
2c	2c	II,V	Pcase IV	A	Penicillins,Carbenicillins	+	-	PSE-1, PSE-3, PSE-4
2d	2d	V	Pcase II, Pcase III	D	Penicillins, Cloxacillin	±	-	OXA-1 to OXA-11, PSE-2 (OXA-10)
2e	2e	1c	Cxase	A	Cephalosporins	+	-	Inducible cephalosporinases from *Proteus vulgaris*
2f	not included	not included	not included	A	Penicillins, Cephalosporins, Carbapenems	+	-	NMC-A from *Enterobacter cloacae*, Sme-1 from *Serratia marcescens*
3	3	not included	not included	B	Most β lactams, including carbapenems	-	+	L1 from *Xanthomonas maltophilia* CcrA from *Bacteroides fragilis*
4	4	not included	not included	ND[c]	Penicillins	-		Penicillinase from *Pseudomonas cepacia*

a Csase, cephalosporinase; PCase, penicillinase; CXase, cefuroxime-hydrolyzing β-lactamase.

b CA, clavulanic acid.

c ND, not determined.

DETECTION OF BETA LACTAMASES[19,30]

Biochemical tests are used to detect β lactamases. β lactamases hydrolyses benzyl pencillin to produce penicilloic acid which was measured. This acid production was measured by

1. Acidometric method: change in pH of an indicator dye is measured.
2. Iodometric method:
3. Chromogenic cephalosporin method:

Normally Nitrocefin is yellow in colour which turns red on production of β-lactamases**.**

β LACTAMASE INHIBITORS[30]

Beta lactamase inhibitors are enzymes that are similar to β lactam antibiotics. They bind either reversibly or irreversibly to beta lactamase, thereby preventing β lactam antibiotics from destruction. They act as suicide bombers, utilizing all available enzymes. Clavulanic acid, Sulbactam and Tazobactam are some of the examples of beta lactamase inhibitors that have gained importance in clinical medicine.

EXTENDED SPECTRUM BETA LACTAMASES (ESBL)

THE OCCURRENCE OF ESBLS

ESBLs are β-lactamases that belong to Ambler class A. They have the ability to produce resistance to the I generation cephalosporin, II generation cephalosporin and III generation cephalosporin, penicillins and aztreonam (but not the carbapenems or cephamycins). These antibiotics are hydrolysed by these enzymes. β-lactamase inhibitors like clavulanic acid inhibit these enzymes[51] and placed under Bush's classification 2be[52].

ESBL's are first reported in Germany in 1983 followed by France in 1985 among Klebsiella spp. ESBL occurs in every part of the world and in most genera of enterobacteriaceae[51]. Generally, ESBLs are β-lactamases which are mediated by the plasmids. They are present most frequently in Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis and other Gram negative bacilli[53]. There are more than 200 different ESBLs reported[52].

EXTENDED SPECTRUM BETA LACTAMASES - DETECTION METHODS

SCREENING OF ESBL

Disc Diffusion method[51, 53, 54]

The disc diffusion methods are the screening test for ESBL production by Escherichia coli, Klebsiella species, and Proteus mirabilis as proposed by CLSI 2014. Laboratories use cefotaxime, ceftazidime, cefpodoxime, ceftriaxone and aztreonam for the screening of ESBL production. More than one of these antibiotics should be

used to improve the sensitivity of the detection. If the screening test is positive, it should be confirmed by phenotypic confirmatory test.

CLSI Recommendations for ESBL Detection[54]

Drugs(cephalosporins)	CLSI (M100-S24) recommended Zone diameter(sensitive)
Cefotaxime	≥27mm
Ceftriaxone	≥25mm
Ceftazidime	≥22mm
Cefpodoxime	≥17mm
Aztreonam	≥27mm

III. ESBL Detection methods recommended by Clinical Laboratory Standard Institute (CLSI) [54].

Phenotypic confirmatory test / Disc Potentiation Test[51, 53, 54].

The CLSI advocates the phenotypic confirmatory test for the detection of production of ESBL by Klebsiella and Escherichia coli which use the cefotaxime or ceftazidime discs (30µg) with or without clavulanate (10µg). Confluent growth of test organism on Mueller Hinton agar shows difference of 5mm along the cephalosporin with clavulanate disc compared to cephalosporin disc alone.

Double disc synergy test[53,54]

In Mueller – Hinton agar plate a lawn culture of the test organism is done on which third generation cephalosporin disc (Ceftazidime, Cefotaxime or Ceftriaxone) and Augmentin are placed 30 mm apart. When zone of inhibition of cephalosporin extends towards Amoxycillin/clavulanate disc, it is taken as positive for production of ESBL.

1. Broth Micro dilution[51]

Disc potentiation test can be done using broth microdilution assays by using ceftazidime (0.25 to128µg/ml), ceftazidime with clavulanate (0.25/4 to128/4 µg/ml), cefotaxime (0.25 to 64µg/ml) and cefotaxime / clavulanate (0.25/4 to 64/4 µg/ml) decrease in MIC of two fold serial dilution of cephalosporin with clavulanate compared to the MIC of cephalosporin alone suggests positive for ESBL production.

COMMERCIAL METHODS AVAILABLE TO DETECT ESBL

Epsilometer-Test for ESBLs[55,56]

Drug impregnated plastic strips are used in which one end contains a gradient of ceftazidime (0.5µg - 32µg/ml) and ceftazidime plus clavulanic acid (0.064-4µg/ml) in a different concentration gradient on the other side, along with a predetermined concentration of clavulanic acid (4µg/ml). The manufacturer recommends about eight – fold reduction in MIC when combined with clavulanate as positive. The sensitivity of this method is reported as 87-100% and the specificity as 100%.

(ii) Vitek ESBL[56,57]

Vitek ESBL cards contain cefotaxime and ceftazidime alone and cephalosporin plus constant concentration of clavulanate. Cards are inoculated in the same manner as that for regular vitek cards. Cards are analysed automatically as soon as the growth in the control well has attained a set threshold. A prefixed decrease in the growth of cefotaxime and ceftazidime plus clavulanic acid wells is compared with the growth in the wells having cefotaxime/ceftazidime alone, indicates positive for ESBL producer. The sensitivity and specificity of the test is more than 90%.

Molecular ESBL detection techniques

Test	Advantages	Disadvantages
DNA Probes	Gene family specific (e.g., TEM or SHV)	Labour intensive, cannot differentiate between ESBLs and non ESBLs, and variants of TEM or SHV
Polymerase chain reaction	Easier in performing, gene family specific(e.g.,TEM or SHV)	cannot distinguish between non ESBLs and ESBLs, and variants of TEM or SHV

Test	Advantages	Disadvantages
Oligotyping	Detects specific TEM variants	Requires oligonucleotide probes that are specific, labourious, not able to detect new variants.
PCR- RFLP	Easy to perform, specific nucleotide changes can be detected.	Nucleotide changes must result in altered restriction site for detection.
PCR- SSCP	Can distinguish between many of SHV variants	Requires special electrophoresis conditions.
Nucleotide sequencing	All variants can be detected, gold standard.	Labourious, difficult technically and to interpret manually.
Real Time PCR	Rapid identification, minimum cross contamination	Expensive, technical skill required

MATERIALS AND METHODS

MATERIALS AND METHODS

This cross sectional study was done for one year and six months from January 2014 to June 2015 to study the bacterial isolates causing asymptomatic bacteriuria in pregnancy at Government Kilpauk Medical College and Hospital, Chennai. Totally 1000 urine samples were collected from 18-40 years of age group among pregnant women and were studied during this period.

INCLUSION CRITERIA

1. All antenatal women without signs and symptoms of infection in the urinary tract.

EXCLUSION CRITERIA

1. Antenatal women who took antibiotics in the past two weeks.
2. Antenatal women with signs and symptoms of infection in the urinary tract.
3. Structural and functional abnormality of the urinary tract.

SAMPLE COLLECTION[6]

A total number of 1000 antenatal women who attended antenatal clinic at Government Kilpauk Medical College and Hospital were included in the study. From the asymptomatic antenatal women, urine specimens were collected by mid-stream clean catch method in a sterile container, which is wide mouthed and covered with tight fitting lids after obtaining informed consent.

SPECIMEN TRANSPORT

Specimens were transported immediately to the microbiology laboratory and processed without delay.

SAMPLE PROCESSING[37, 58]

Macroscopic examination

Colour, turbidity and deposits of the urine samples were observed macroscopically and the results were recorded[37, 58].

The collected mid stream clean catch urine sample was divided into two portions. One half was taken for urine microscopy and culture and the other half was used for commercial reagent strip test for the nitrite and leucocyte esterase. The presence of nitrite and leucocyte esterase is noted by specific colour change in the panel provided by the manufacturer. The samples were tested as per the manufacturer's instructions. The results were compared with the colour code on the reagent strip container between 30 and 60 seconds as per the instruction manual. The positive results were recorded[59].

Microscopic examination

1. **PUS CELL COUNT OF THE UNCENTRIFUGED URINE[60]**

   Presence of polymorphonuclear neutrophils (PMNs) was detected by urine wet mount examination. Microscopic analysis was done with 0.05 ml of the uncentrifuged urine.

   ≥ 5 pus cells/high power field - positive finding.

2. GRAM STAIN OF UNCENTRIFUGED URINE[37,61]:

One drop of uncentrifuged urine was smeared on a grease free glass slide. It was air dried, heat fixed, Gram stained and seen under oil immersion objective. Significant bacteriuria correlates with the presence of atleast 1 organism / oil immersion field on examining 20 fields.

URINE CULTURE

Semi-quantitative method[37, 40, 61]

To isolate the organism, a semi-quantitative calibrated loop technique was used. One loopful of properly mixed urine that was not centrifuged was inoculated onto the surface of Nutrient agar, 5% sheep Blood agar, Mac Conkey agar and Cysteine Lactose Electrolyte Deficient agar using a calibrated loop that delivers 0.01 ml of urine sample. The culture plates were incubated under aerobic conditions at 37^0 C for 18-24 hours. The colonies were counted using colony counter and the number of colony forming units were multiplied by 100 to find out the number of microorganisms present per millilitre of urine.

$\geq 10^5$ colony forming units/ml - significant bacteriuria.

The diagnostic criteria for asymptomatic bacteriuria was considered when atleast two consecutive urine samples showed more than or equal to 10^5 colony forming units in 1ml of urine of the single species without any UTI symptoms[62]. To differentiate pathogens from commensals, standard microbiological methods were followed. The importance of the isolates were identified based on presence of pus cells in direct

Gram staining, pure growth and colony morphology on culture plates and biochemical reactions by using standard microbiological techniques.

VIRULENCE FACTORS OF VARIOUS ORGANISMS

VIRULENCE FACTORS OF ESCHERICHIA COLI

Haemolysin - Plate haemolysis test[63]:

The alpha haemolysin production by Escherichia coli was tested by the plate haemolysis test. On 5% sheep blood, the bacteria was inoculated and incubated at 35°C overnight. A zone of lysis of the red blood cells around the colony and clearing of the medium indicates haemolysin production.

The hemolysin detection was done for various other organisms like Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis.

The gelatinase test[19]:

Using gelatin agar, the gelatinase production was tested. The organism was inoculated on to the plate and kept for incubation at 37° C aerobically for 24 hours. 1% tannic acid solution was poured over the colonies. The test was considered positive if there was a development of opacity around the gelatin liquefying colonies, quick to develop but fades as the medium also becomes opaque.

Cell surface hydrophobicity[63]

Salt aggregation test (SAT) was used to determine the cell surface hydrophobicity of Escherichia coli. Using phosphate buffer a bacterial suspension was prepared. 10µL of the suspension and 10 µL of ammonium sulphate solution of

differing molarity, i.e., from 0.3125 M through 5 M was mixed on a glass slide and rotated for 1 minute and observed.

Salt aggregation test was considered positive if there was visible clumping in highest dilution.

Auto Aggregative Strains – Clumping in 0.002 M phosphate buffer alone. Escherichia coli strains which had a Salt Aggregation Test value of ≤ 1.25 M were taken as cell surface hydrophobicity positive.

Serum resistance[63]

Overnight cultures of Escherichia coli grown on blood agar at 37°C were taken and mixed in Hank's balanced salt solution (HBSS). Fresh human serum (0.05 ml) and (0.05 ml) of bacterial suspension was mixed and incubated at 37° C for 3 hours. 10µL of samples were then inoculated on blood agar plates and incubated for 18 hours at 37° C and viable count calculated. The percentage of bacteria viable after 3 hours of incubation indicates the resistance of bacteria to serum bactericidal activity.

Serum sensitive: viable count decreased to 1% of previous value.

Serum resistant: viable count more than 90% of organisms survived after 3 hours.

Haemagglutination test[64]

The test strain was taken from the Nutrient agar plate to peptone water (pH 7.0) and grown at 37^0C for 24 hours. Human'O'erythrocytes were taken in Alsever solution and were washed two times with phosphate buffered saline (PBS) and 3%

suspension was made in the same buffer. One drop of bacterial culture and one drop of erythrocyte suspension were carefully mixed on a slide. The slide was rotated for 1 minute at room temperature and the presence and absence of macroscopic haemagglutination was seen. To test the effect on haemagglutination by D-mannose, 1 drop of mannose solution (25mg/ml of PBS; Ph 6.8) was added prior to the addition of red blood cell suspension and any difference in the degree of haemagglutination was noted.

BIOFILM DETECTION BY MICROTITRE PLATE METHOD[65]

From fresh agar plates the organisms were taken and inoculated into Brain Heart Infusion Broth with 2% sucrose and kept in the incubator at 37^0 C for 24 hours. With fresh medium, broth was diluted to 1: 100. 200 microlitres of the diluted cultures were inoculated into a sterile 96 well flat bottomed culture plate made of polystyrene. Medium without culture was used as control to look for the sterility and the non specific binding of the media. At 37^0 C, the microtitre plate was kept in the incubator for 24 hours.

At the end of incubation the plates were tapped to remove the contents of each well. The wells were then washed with 200 µl of phosphate buffer saline for 4 times to eradicate the planktonic bacteria. Biofilms produced by sessile adherent bacteria in plate were stained with 0.1% crystal violet for one minute after fixing with 2% sodium acetate. Excess stain was washed thoroughly with water without any ions and the plate was dried in air. Adherent organisms which produced biofilm on all side of the wells were evenly stained with crystal violet.

Optical density (OD) of adherent bacteria was determined with a micro ELISA auto reader at 570 nm wavelength. These OD values represent the bacteria strongly attached to surface and biofilm producing. Experiment was done in triplicate and the values were then averaged and standard deviation (SD) was calculated. The average OD value of the media control well was subtracted from all the test values.

Classification of bacteria adherence:

The reading values are interpreted as follows

Sample OD Value	**Biofilm Formation**
Sample OD > 0.24	Strong biofilm producers
Sample OD values between 0.12-0.24	Moderate biofilm producers
Sample OD < 0.12	Non/weak biofilm producers.

The biofilm detection was done for various other organisms like Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis.

MANNOSE RESISTANT HAEMAGGLUTINATION GENE DETECTION BY POLYMERASE CHAIN REACTION (PCR) [16, 66, 67]

Methods for DNA Extraction:

DNA extraction was performed with DNA purification kit and by using Polymerase chain reaction master mix.

Master Mix 2X has 2U of Taq DNA polymerase, 10X Taq reaction buffer, 2mM Magnesium chloride and 1µl of 10mM dNTPs mix, Polymerase Chain Reaction additives. For performing gel electrophoresis the following were used.

Agarose, 50X TAE buffer, 6X gel loading buffer, Ethidium bromide.

DNA extraction Procedure:

The overnight culture of the Escherichia coli in nutrient broth was taken in an amount of 1.5 ml. It was then centrifuged for 5 minutes at 6000 rotations per minute. Then the sediment was taken as the pellet.

- In 200µl of Phosphate Buffer Saline (PBS) pellet was suspended.
- 180µl of lysozyme Digestion Buffer was mixed to 20 microlitre of lysozyme and incubated at 37°C for 15 minutes.
- 200 microliters of Lysis buffer was mixed gently with 20µl of Proteinase K [10mg/ml] and incubated in a water bath at 56°C for ten minutes
- 300 microlitre of isopropanol was added and mixed well.
- Whole lysate was transferred into Pure Fast spin column. It was then centrifuged for one minute at 10000 rpm.
- Discard flow through and 500µl of Wash Buffer-1 was added and centrifuged at 10000 rpm for 1Minute.
- Discard flow through and 500µl of Wash Buffer-2 was added and centrifuged at10000 rpm for 1minute. This procedure was done for two times.
- Discard flow through centrifuged column for 2 more minutes so that any residual ethanol will be removed.
- 100µl of Elution Buffer was added to elute the DNA, and centrifuged for one minute.

Extracted DNA Quality and Quantity was verified by loading in 1% agarose gel with 10µL of DNA extracted.

Primer

Pap A-145bp

Forward Primer: 5'- GGCGCTGACAGAAGGTGCCATT- 3'

Reverse Primer: 5'- CATGCCCAGTTCCCGGCCTTTT- 3'

PCR Product size: 145bp

Procedure for running PCR:

[25µl of Master Mix: 10X Taq buffer, 2mM Magnesium chloride, 0.4mM dNTPs mix, and 2U *Proofreading* Taq DNA polymerase]

1. Reactions were set up as;

Components/Quantity

Master Mix in PCR vial -10µl

Primer Mix (10pmoles/µl) - 5µl

Genomic DNA - 5µl

Total amount - 20µl

2. All these were mixed gently and spinned down briefly.

3. They were then placed in the PCR machine and programmed;

Initial Denaturation: 94ºC for 5 minutes

Denaturation: 94ºC for 30seconds in cycles of 35

Annealing: 58ºC for 30 seconds in cycles of 35

Extension: 72ºC for 30 seconds in cycles of 35

Final extension: 72º C for 5 minutes

Loading:

1. Agarose gel 2% was made. [In 100ml of 1x TAE buffer 2gm of agarose was mixed]
2. 8µl 6X Gel loading dye was taken in each PCR vial and 15µl of polymerase chain reaction sample was loaded.
3. At 50V, electrophoresis was run till the dye reaches 3/4th distance and the bands were seen in UV Transilluminator.

Method to perform Agarose gel electrophoresis:

- 2gm agarose was mixed in 100ml of 1X TAE buffer; it was melted by heating in a micro oven.
- When the temperature of agarose gel was about 60ºC, Ethidium bromide (5µl) was added.
- Into the gel platform warm agarose solution was added slowly.
- Till the agarose got solidified it was kept undisturbed.
- Into the submarine gel tank1XTAE buffer was added.
- Gel platform was kept into tank without any disturbance. The tank buffer level was kept maintaining upto 0.5cm above the gel.
- Polymerase Chain Reaction samples were loaded after mixing with gel loading dye and 10µl HELINI 100bp DNA Ladder.
- Electrophoresis was done at 50V till the dye reaches 3/4th distance of the agarose gel. The Agarose gel was seen with UV Transilluminator, the pattern of the bands were seen and interpreted.

VIRULENCE FACTORS FOR KLEBSIELLA PNEUMONIAE:

Demonstration of Capsule[19]:

A large loopful of undiluted India ink was taken on a slide and a small amount of solid bacterial culture was emulsified. A clean, grit free coverslip was placed on the slide. The capsule was seen as a clear zone surrounding the microorganism on the brown-black ground.

VIRULENCE FACTORS FOR PROTEUS MIRABILIS[33, 68]

The virulence factors of Proteus mirabilis were done by hemolysin detection, serum resistance, mannose resistant haemagglutination and biofilm detection.

VIRULENCE FACTORS OF PSEUDOMONAS AERUGINOSA

Virulence factors of Pseudomonas aeruginosa was determined by β-hemolysin detection[30], pyocyanin pigment seen in Nutrient agar plate[69, 70] and biofilm detection.

VIRULENCE TESTS FOR STAPHYLOCOCCUS AUREUS

Slide coagulase test[30]

A colony of the test organism was emulsified with saline on a microscopic glass slide. Similar suspensions of positive and negative control strains were used. One drop of undiluted plasma was mixed to the test suspension on the slide and mixed with the sterile applicator stick. The slide was rocked back and forth. A coarse clumping of the organism visible to the naked eye within 10 seconds was taken as positive.

Tube coagulase test[19]

A 1-in-6 dilution of the plasma in 0.85% NaCl was taken and 1 ml volumes of it were taken in small tubes. A colony of the suspected organism was emulsified in a tube of the diluted plasma. With each batch of tests, tubes with known coagulase positive and coagulase negative cultures were used as controls. A tube of unseeded diluted plasma was also used to check that the plasma does not clot spontaneously.The tubes were kept in a water-bath at 37^0C for 4 hours and were examined at 1, 2 and 4 hours for clot formation by slanting the tube through 90 degrees. Any degree of clot formation was taken as positive.

Urease production[19]

Christensen's urea slopes were used. The slope was inoculated heavily with the organism over the entire slope surface and incubated at 37^0C. The slope was examined after 4 hours and after overnight incubation. The slopes were considered as urease positive if the colour changed to purple-pink.

DNase test[19]

The test was done using DNase agar. On the DNase agar spot inoculation of the test organism were done and incubated at 37^0C for 18-24 hours aerobically. The plate was then flooded with a few ml of 1 mol/litre (3.6%) hydrochloric acid. Unhydrolysed DNA was precipitated and which produced a white opacity or cloudiness in the agar was considered as positive.

Phosphatase test[19]

1% aqueous solution of sodium phenolphthalein diphosphate was prepared and 10 ml of it was added to 1000 ml of melted Nutrient agar cooled to 50^0C and the plates were poured. On this medium, the test organism was grown overnight at 37^0C. The plate was inverted and a few drops of ammonia solution SG 0.88 were added. The colonies which turned bright pink within a few minutes were considered as positive.

VIRULENCE FACTORS OF ENTEROCOCCI[71]

The virulence factors of enterococci were the gelatinase, β-hemolysin and the biofilm production.

Gelatinase test[71]

Enterococus isolates were inoculated onto peptone yeast extract agar having gelatin (30g/L) and kept in the incubator for 24 hours at 37^0 C. The plates with the growth were then cooled for two hours at ambient temperature. Gelatinase production was indicated by the appearance of a turbid halo around the colonies.

ANTIBIOTIC SUSCEPTIBILITY TESTING[72, 73]:

Antibiotic sensitivity testing was performed by the Kirby-Bauer's disc diffusion method using Mueller Hinton agar as per CLSI guidelines 2014(M100-S24).

The quality control strains used were

Staphylococcus aureus - ATCC 25923

Escherichia coli - ATCC 25922

Pseudomonas aeruginosa - ATCC 27853.

TURBIDITY STANDARD FOR INOCULUM PREPARATION

For a susceptibility test, density of the inoculum was standardized by a BaSO4 turbidity standard, which was equal to 0.5 McFarland standards or its optical equivalent like latex particle suspension was used.

Inoculum preparation

In a sterile test tube isolated colonies (approx. 3-5) belonging to similar morphological type was taken and mixed in about 4-5 ml of a suitable broth medium. The broth culture was incubated at 35˚C until it attained 0.5 McFarland turbidity medium. This suspension corresponds to 150 million organisms/ml.

Method of inoculation of test plates

The turbidity of the test medium was adjusted and after that it was inoculated within 15 minutes into the plates. A sterile cotton swab is immersed into the suspension and it is pressed along the sides of the tube. The excess inoculum from the swab was removed like this. The swab was then streaked onto a previously dried Mueller Hinton agar plate. This procedure was repeated 2-3 times by rotating the plate 60˚ so that the inoculum got evenly distributed onto the plate. Finally, the rim of the plate was swabbed. Within 15 minutes, the antibiotic discs were impregnated onto the plate. The discs were pressed firmly to confirm complete contact with the surface of the agar. It was ensured that the discs are placed 24mm from center to center. The plate was inverted and kept in the incubator within 15 minutes after the application of the drugs. The plate was examined after 16-18 hours of incubation. The zone of

inhibition was uniformly circular with a confluent lawn of growth. The diameter of the inhibition zone was calculated with the help of sliding calipers or rulers and interpreted as per CLSI guidelines

TESTS TO DETECT METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

CEFOXITIN DISC DIFFUSION TEST[74]

The test was performed by placing 30µg of cefoxitin disc in the Mueller Hinton Agar plate without NaCl supplementation inoculated with test organism. The plate was kept in the incubator at a temperature of 37˚C. The zone of inhibition was determined after 24 hrs and the zone size was interpreted as

Susceptible – ≥ 20 mm

Resistant – ≤ 19 mm

METHODS TO DETECT EXTENDED SPECTRUM BETA LACTAMASES[51, 53, 54]

Quality control

Quality controls were performed using

Escherichia coli ATCC 25922 - Negative control

Klebsiella pneumoniae ATCC 700603 - Positive control.

Disk diffusion methods

Disk diffusion test was done for all Enterobacteriaceae isolates against cefotaxime (30 μg), ceftriaxone (30 μg), cefpodoxime (10 μg) and ceftazidime (30 μg) antibiotic disks for the screening of the isolates for potential ESBL production. Overnight incubation was done at 37˚C after which the zone size was read as per CLSI recommendations for ESBL screening criteria.

Phenotypic confirmatory tests or disc potentiation test[75, 76, 77, 78]

This test was done for all enterobacteriaceae isolates against Ceftazidime (30 μg) antibiotic discs with and without clavunalic acid (10 μg). These discs were placed on a Mueller – Hinton agar plate inoculated with bacterial suspension equivalent to 0.5 McFarland standards. Overnight incubation was done at 37°C after which the result was interpreted as follows:

If the zone diameter of ceftazidime / clavulanic acid was augmented by ≥ 5 mm in comparison with ceftazidime alone was taken as ESBL positive.

Detection of ESBL Producers using E-test ESBL strip[79, 80]

The ESBL E-strip was based on two gradients. One end of the strip contained ceftazidime (0.5-32 μg/ml), and the opposite end was impregnated with ceftazidime (0.125-8μg/ml) and clavulanate (4μg/ml). An overnight culture of the test organism on brain – heart infusion agar was suspended in saline to match the 0.5

McFarland standard turbidity. Then the suspension was used to inoculate a Mueller Hinton agar plate by swabbing the plate using a sterile cotton swab. The E-strip was placed on the plate after the plate was dried and it was incubated at 37^0 C overnight.

For both the ends of the strip, the point of intersection between the inhibition eclipse and the edge of the E-strip was considered the minimum inhibitory concentration (MIC). A ceftazidime MIC / ceftazidime-clavulanate MIC ratio ≥ 8 indicates the presence of ESBL enzymes as per the manufacturer's instruction manual.

DETECTION OF ESBL GENE BY POLYMERASE CHAIN REACTION (PCR)[81]

Methods for DNA Extraction:

DNA extraction was performed with DNA purification kit and by using Polymerase chain reaction master mix.

Master Mix 2X has 2U of Taq DNA polymerase, 10X Taq reaction buffer, 2mM Magnesium chloride, 1µl of 10mM dNTPs mix and Polymerase Chain Reaction additives.

Agarose gel electrophoresis: Requirements

Agarose, 50X TAE buffer, 6X gel loading buffer, Ethidium bromide.

DNA extraction Procedure:

The overnight culture of the Escherichia coli and Klebsiella pneumoniae were taken in nutrient broth in an amount of 1.5 ml. It was then centrifuged for 5 minutes at 6000 rotations per minute. Then the sediment was taken as the pellet.

- In 200µl of Phosphate Buffer Saline (PBS) pellet was suspended.
- 180µl of lysozyme Digestion Buffer was mixed to 20 microlitre of lysozyme and incubated at 37°C for 15 minutes.
- 200 microliters of Lysis buffer was mixed with 20µl of Proteinase K [10mg/ml] gently, and kept in the water bath at 56°C for ten minutes
- 300 microlitre of isopropanol was added and mixed well.
- Whole lysate was transferred into Pure Fast spin column. It was then centrifuged at 10000 rpm for one minute
- Discard flow through and 500µl of Wash Buffer-1 was added and centrifuged at 10000 rpm for 1Minute.
- Discard flow through and 500µl of Wash Buffer-2 was added and centrifuged at10000 rpm for 1minute. This step was done twice.
- Discard flow through centrifuged column for 2 more minutes so that any residual ethanol will be removed.
- 100µl of Elution Buffer was added to elute the DNA, and then centrifuged for one minute.
- Extracted DNA Quality and Quantity was verified by loading in 1% agarose gel and 10µL of DNA extracted was used by polymerase chain reaction for amplification.

Primer

CTX-M-14

Forward Primer: 5'- TTATGCGCAGACGAGTGCGGTG - 3'

Reverse Primer: 5'- TCACCGCGATAAAGCACCTGCG - 3'

PCR Product size: 120bp

Procedure for PCR:

[25µl of Master Mix has: 10X Taq buffer, 2mM Magnesium chloride, 0.4mM dNTPs mix, and 2U *Proofreading* Taq DNA polymerase]

1. Reactions done as follows;

Components with quantity:

Master Mix in PCR vial-10µl

Primer Mix (10pmoles/µl) - 5µl

Genomic DNA - 5µl

Total volume - 20µl

2. All these are mixed gently and spinned down briefly.

3. Placed into polymerase chain reaction machine and programmed as follows;

Initial Denaturation: 94ºC for 5 minutes

Denaturation: 94ºC for 30 seconds - 35 cycles

Annealing: 58ºC for 30 seconds - 35 cycles

Extension: 72ºC for 30seconds - 35 cycles

Final extension: 72º C for 5 minutes

Loading:

4. 2% agarose gel was made by mixing 2gm of agarose in 100ml of 1x TAE buffer
5. To 8µl 6X Gel loading dye 15µl of PCR sample was added in PCR vial.
6. At 50V electrophoresis was run till the dye reaches 3/4th distances and the bands were observed in UV Transilluminator.

Method to perform Agarose gel electrophoresis:

- 2% agarose was made by mixing 2gm agarose in 100ml of 1X TAE buffer; it was melted by heating in a microoven.
- 5µl of Ethidium bromide was added when the agarose gel temperature was about 60ºC.
- Agarose solution which was warm was poured gently into the gel platform.
- The gel was not disturbed till the agarose got solidified.
- Into submarine gel tank 1XTAE buffer was added.
- Gel platform was kept into tank without disturbance. The tank buffer was maintained upto 0.5cm above the gel.
- Polymerase Chain Reaction samples were loaded and after that they were mixed with gel loading dye and along with 10µl HELINI 100bp DNA Ladder.
- At 50V, electrophoresis was performed till the dye reaches 3/4th distance of the agarose gel.
- The Agarose gel was seen with UV Transilluminator; the bands were seen and interpreted.

STATISTICAL ANALYSIS

The test outcome was observed, recorded and analysed. The data that were analysed was presented in the form of statistical tables, pie charts and histograms if necessary in appropriate places. P values were calculated by Pearson Chi-Square and Fishers exact Chi-Square test. The data were documented and studied in detail. The documented data was further discussed in detail and compared with other similar studies published in reputed scientific journals.

RESULTS

RESULTS

Antenatal women attending Obstetrics OP at Government Kilpauk Medical College and Hospital, Chennai were included in the study for asymptomatic bacteriuria detection. The isolates were studied for their virulence factors, Extended Spectrum Beta Lactamases (ESBL) and Methicillin Resistant Staphylococcus aureus (MRSA). The study was done between 'January 2014 to June 2015'. 1000 Antenatal women without signs and symptoms of infection of the urinary tract were tested and 118 bacterial isolates were isolated, identified, their virulence factors were tested and analysed for their antibiotic sensitivity pattern. Resistant isolates were identified and studied for Extended Spectrum Beta Lactamases (ESBL) and Methicillin Resistant Staphylococcus aureus (MRSA) by various methods.

The observations were recorded and analysed. The results were as follows:

TABLE-1 Asymptomatic bacteriuria in antenatal women (n=1000)

ASYMPTOMATIC BACTERIURIA IN ANTENATAL WOMEN	
Total number of Antenatal women	**Antenatal women with Asymptomatic bacteriuria**
1000	118(11.80%)

Out of the 1000 urine samples, 118 (11.8%) were culture positive.

TABLE – 2 AGE WISE DISTRIBUTION (n=118)

Age in years	Culture Positives
18- 20	28(23.73%)
21- 30	78(66.10%)
31- 40	12(10.17%)
Total	118(100%)

Of the 118 isolates, the asymptomatic bacteriuria was high in the age group of 21-30 years 78(66.10%).

TABLE – 3 RELATIONSHIP OF PARITY AND CULTURE POSITIVES (n=118)

Gravida	Culture Positives
Primi gravida	58(49.2%)
Second gravida	41(34.7%)
Third gravida	18(15.3%)
Gravida more than three	1(0.8%)
Total	118(100%)

Out of 118 bacterial isolates, the culture positives were more in the Primigravida 58(49.2%).

CHART - I

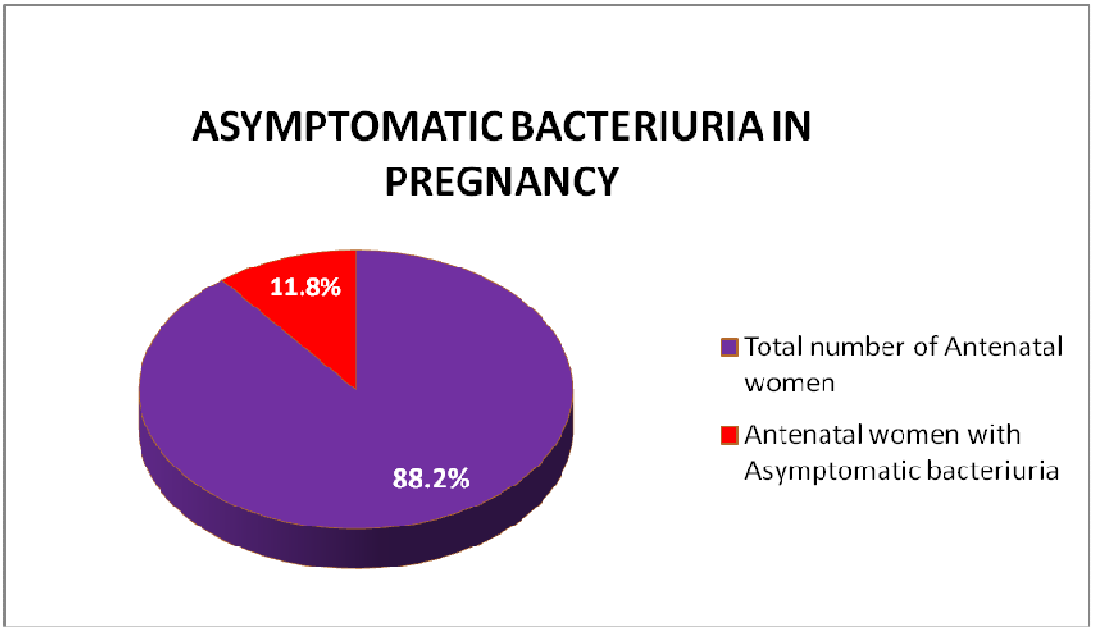
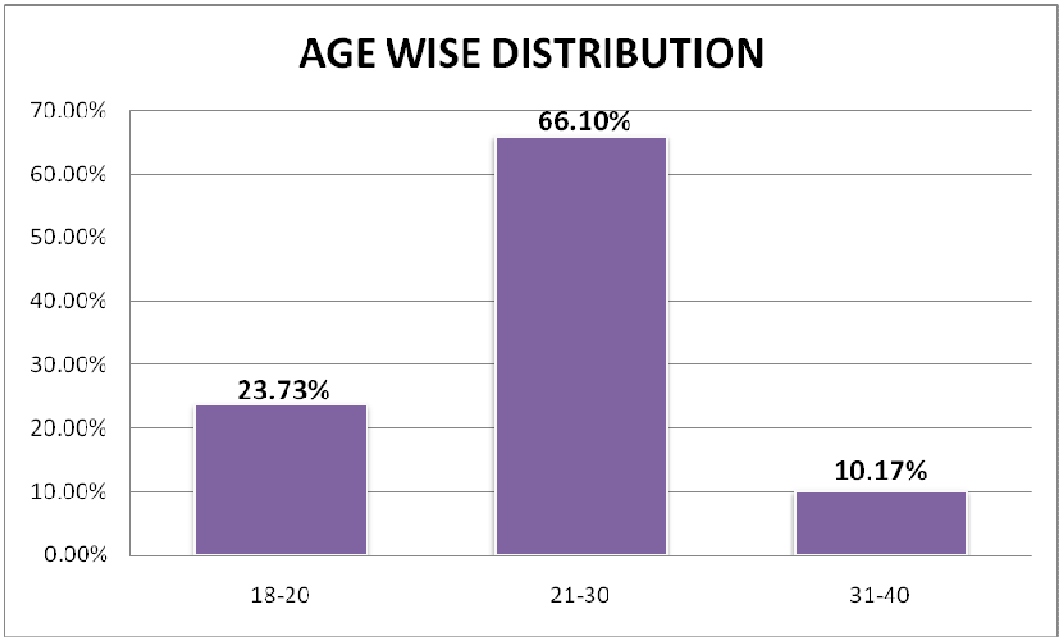


CHART - II



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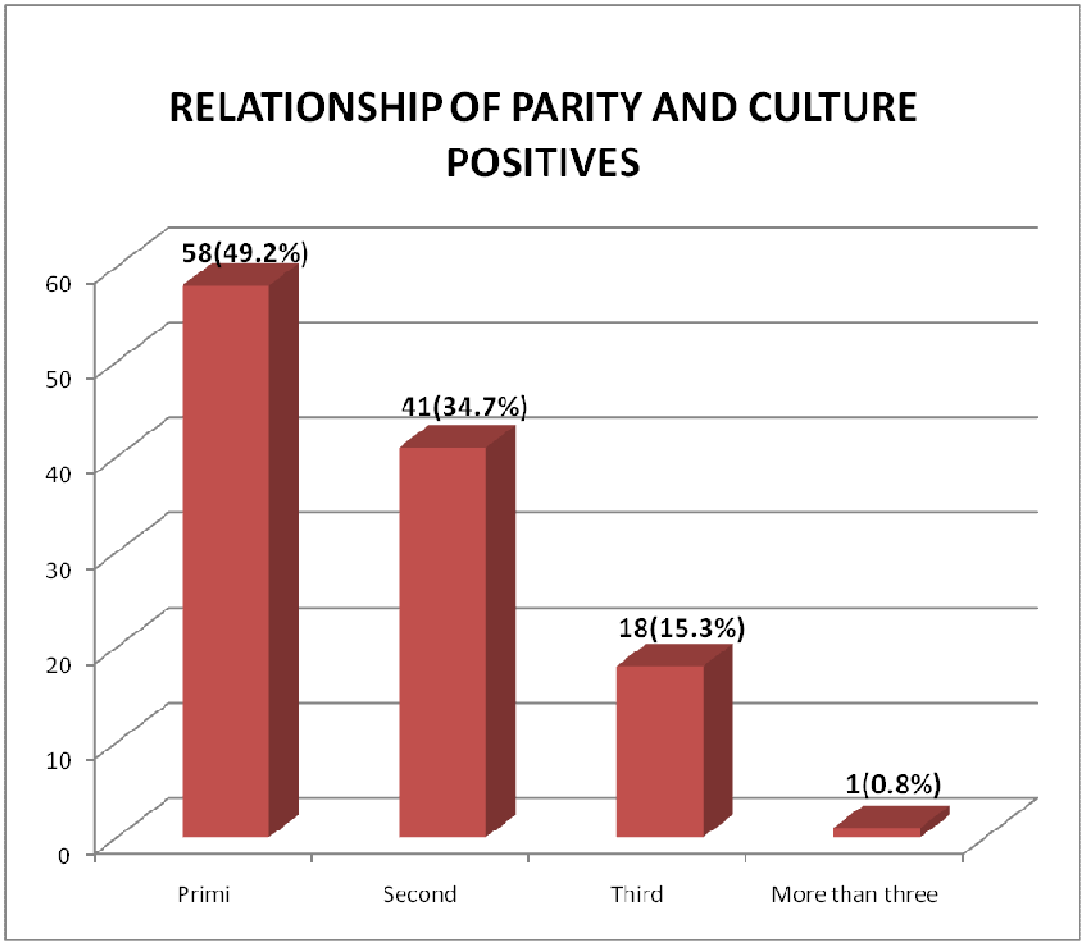

RELATIONSHIP OF PARITY AND CULTURE POSITIVES
58(49.2%)
41(34.7%)
18(15.3%)
1(0.8%)
60
50
40
30
20
10
0
Primi
Second
Third
More than three

TABLE -4 RELATIONSHIP OF DURATION OF PREGNANCY AND CULTURE POSITIVES (n=118)

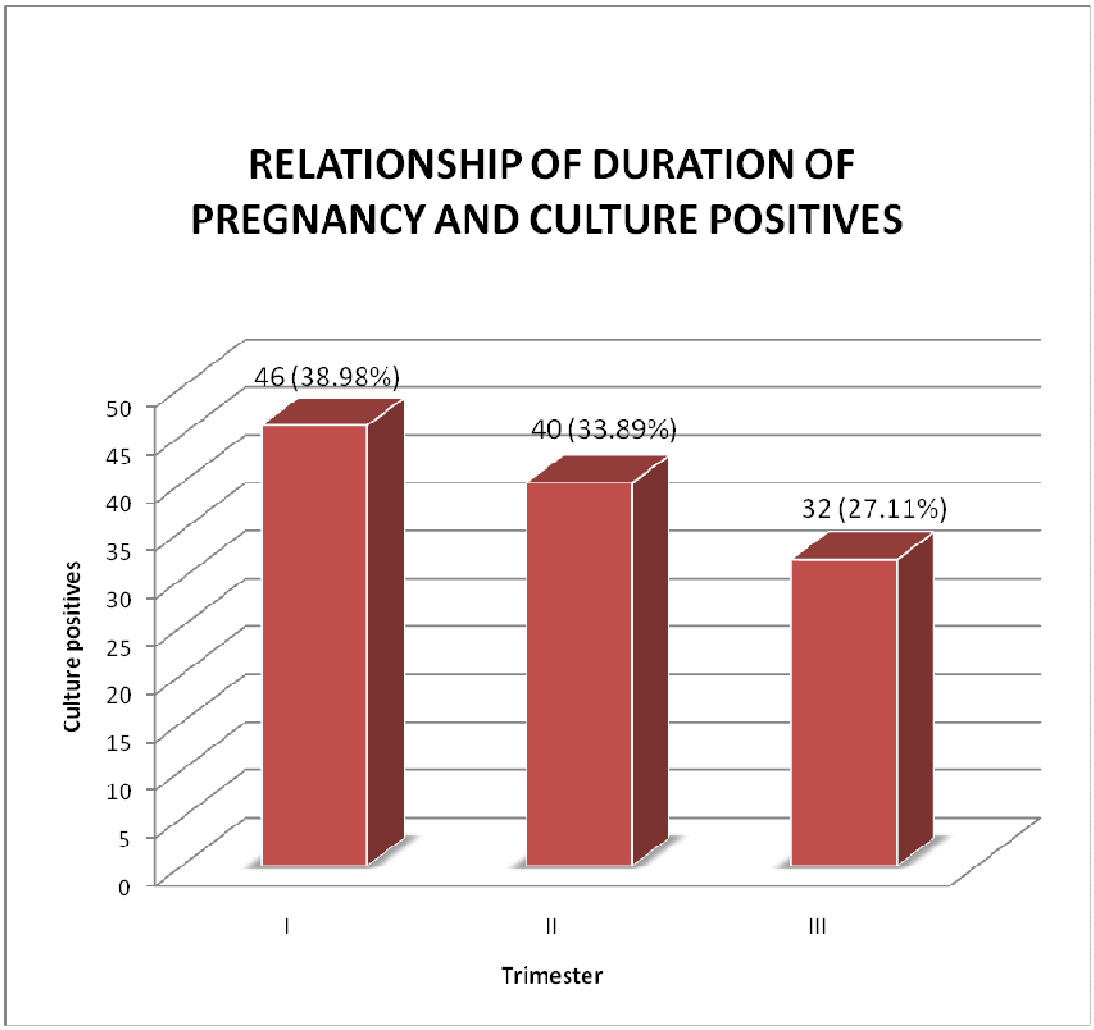
Trimester	Culture positives
I	46(38.98%)
II	40(33.89%)
III	32(27.11%)
Total	118(11.8%)

Out of the 118 bacterial isolates the culture positives were more in the first Trimester 46(38.98%)

TABLE NO.5 SCREENING TESTS FOR ASYMPTOMATIC BACTERIURIA IN PREGNANCY (n =1000)

	PUS CELLS		DIRECT GRAM STAIN SMEAR		NITRATE REDUCTION		LEUCOCYTE ESTERASE	
Culture Positivity	Positive	Negative	Positive	Negative	Reduced	Not Reduced	Positive	Negative
Culture Positive	67	51	93	25	89	29	71	47
Culture Negative	30	852	9	873	11	871	36	846

CHART - IV


RELATIONSHIP OF DURATION OF PREGNANCY AND CULTURE POSITIVES
46 (38.98%)
40 (33.89%)
32 (27.11%)
Culture positives
50
45
40
35
30
25
20
15
10
5
0
I
II
III
Trimester

Out of the 118 culture positives, 67 had significant pus cells, 93 had organisms in Gram stain, 89 reduced nitrate and 71 were positive for leucocyte esterase by urine dipstick. In our study there was statistical significance for all the four screening tests with the p value of ($p < 0.001$)

TABLE –6 GRAM NEGATIVE BACILLI VS GRAM POSITIVE COCCI (n=118)

ORGANISM	PERCENTAGE
Gram Negative Bacilli	78(66.10%)
Gram Positive Cooci	40(33.90%)

Out of the 118 isolates, total number of Gram negative bacilli were 78(66.10%) and total number of Gram positive cocci were 40(33.90%)

TABLE – 7 DISTRIBUTION OF ORGANISMS (n=118)

ORGANISMS	PERCENTAGE
Escherichia coli	54(45.76%)
Staphylococcus aureus	21(17.80%)
Klebsiella pneumoniae	19(16.10%)
Staphylococcus saprophyticus	10(8.45%)
Enterococcus faecalis	9(7.63%)
Pseudomonas aeruginosa	3(2.54%)
Proteus mirabilis	2(1.69%)

CHART - V

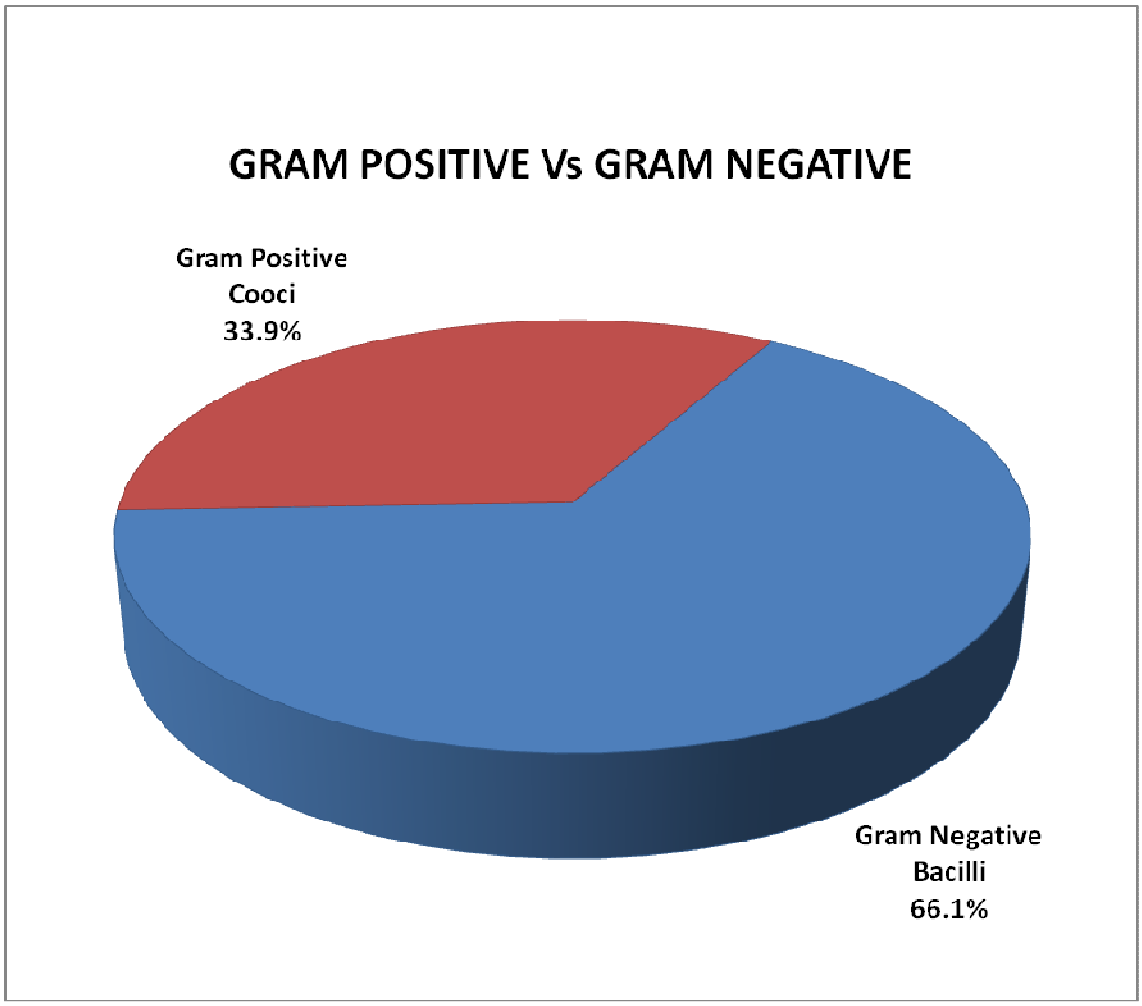
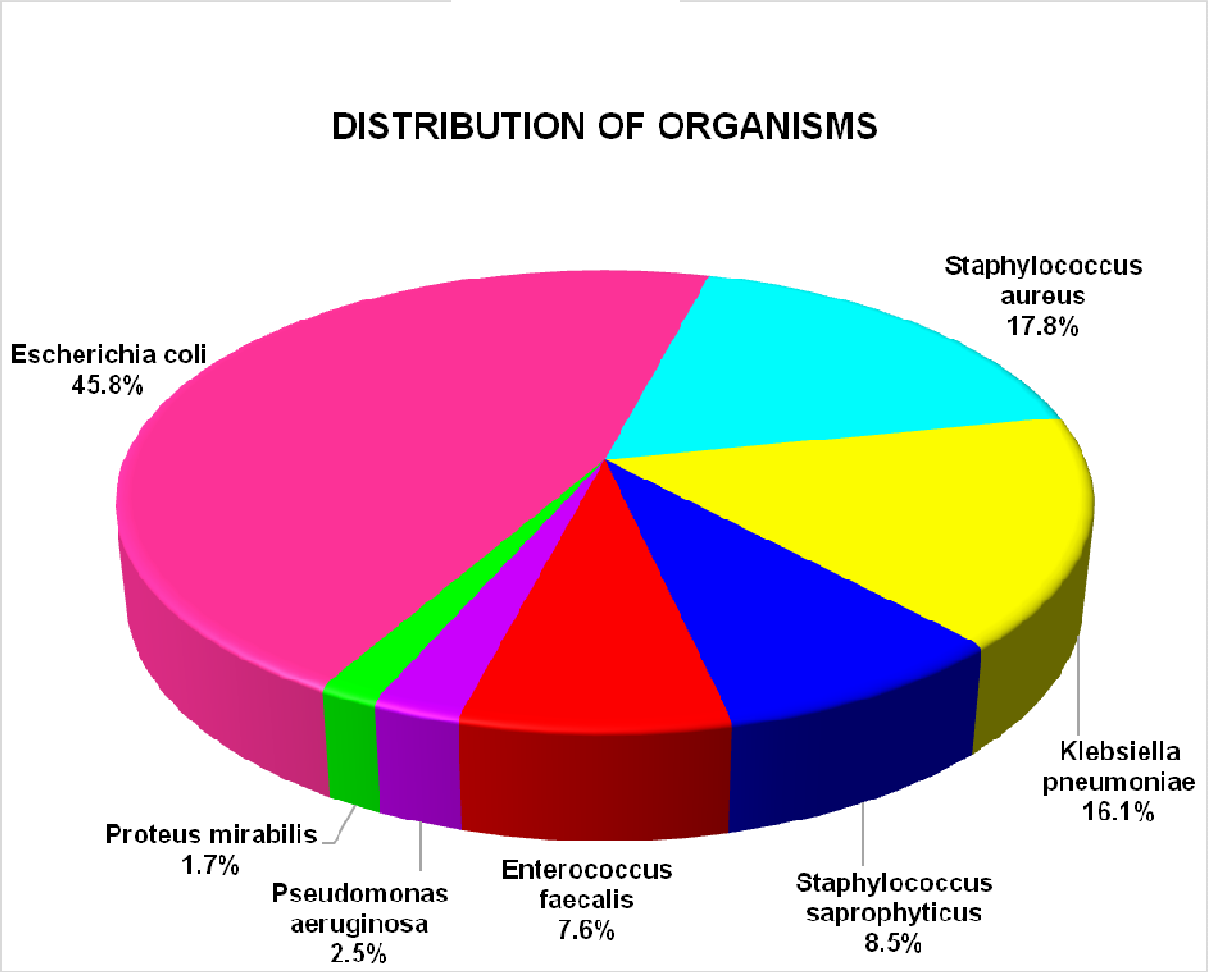

GRAM POSITIVE Vs GRAM NEGATIVE
Gram Positive
Cooci
33.9%
Gram Negative
Bacilli
66.1%

CHART - VI


DISTRIBUTION OF ORGANISMS
Staphylococcus aureus 17.8%
Escherichia coli 45.8%
Klebsiella pneumoniae 16.1%
Proteus mirabilis 1.7%
Pseudomonas aeruginosa 2.5%
Enterococcus faecalis 7.6%
Staphylococcus saprophyticus 8.5%

Out of the 118 isolates isolated from 1000 antenatal women, Escherichia coli 54(45.76%) was the predominant isolate followed by Staphylococcus aureus. Proteus mirabilis was the least common organism isolated 2(1.69%).

TABLE-8 A

VIRULENCE FACTORS OF ESCHERICHIA COLI (n=54)

Tests for virulence	Virulent Isolates
Hemolysin	18(33.33%)
Cell surface hydrophobicity	11(20.37%)
Serum resistance	11(20.37%)
Gelatinase test	10(18.51%)
Mannose Resistant Haemagglutination	9(16.66%)
Biofilm producers	6(11.11%)

Out of the 54 Escherichia coli isolates, 9(16.66%) were mannose resistant haemagglutination positive, 18 (33.33%) were Hemolysin positive, 11(20.37%) were Cell Surface Hydrophobicity and Serum Resistance positive. The p-value for gelatinase ($p=0.042$), serum resistance ($p=0.011$), biofilm ($p=0.022$) and cell surface hydrophobicity ($p<0.001$) were statistically significant with p value < 0.05.

CHART - VII

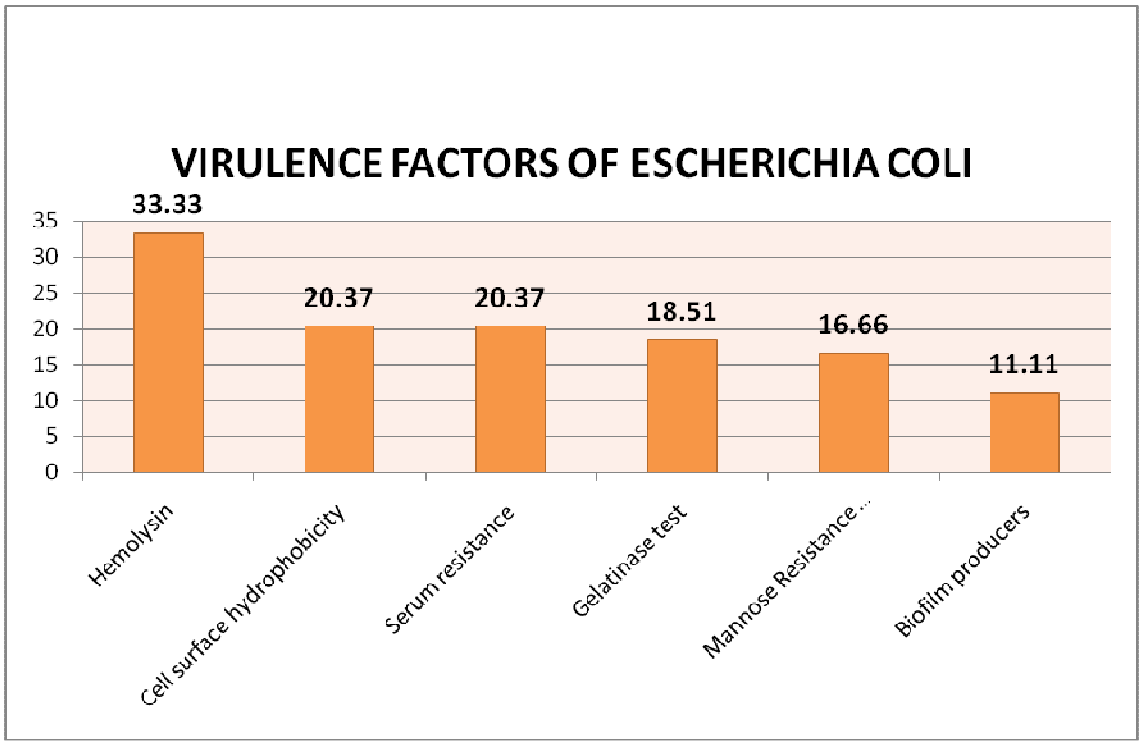

VIRULENCE FACTORS OF ESCHERICHIA COLI
35
30
25
20
15
10
5
0
33.33
20.37
20.37
18.51
16.66
11.11
Hemolysin
Cell surface hydrophobicity
Serum resistance
Gelatinase test
Mannose Resistance ...
Biofilm producers

TABLE-8 B

MULTIPLE VIRULENCE FACTORS OF ESCHERICHIA COLI (n=54)

Name of the virulence Factors	No. of Virulent Isolates
Mannose Resistant Haemagglutination + Serum resistance + Cell surface hydrophobicity	1
Cell surface hydrophobicity+ Serum resistance+ Gelatinase	1
Serum resistance+ Gelatinase+ Biofilm producers	1
Hemolysin+ Mannose Resistant Haemagglutination + Serum resistance	1
Mannose Resistant Haemagglutination+ Gelatinase+ Biofilm producers	1
Hemolysin+ Cell surface hydrophobicity	4
Mannose Resistant Haemagglutination+ Gelatinase	1
Hemolysin+ Biofilm producers	3
Hemolysin+ Serum resistance	1
Hemolysin+ Mannose Resistant Haemagglutination	2
Cell surface hydrophobicity + Gelatinase	2
Hemolysin+ Gelatinase	3
Cell surface hydrophobicity+ Serum resistance	2
Serum resistance + Biofilm producers	1
Hemolysin Only	4
Mannose Resistant Haemagglutination Only	3
Cell surface hydrophobicity Only	0
Serum resistance Only	4
Gelatinase test Only	1
Biofilm producers Only	0
No Virulence Factors	18

Out of the 54 Escherichia coli isolates, 5 isolates had 3 virulence factors positive, 19 isolates had 2 virulence factors positive and 12 isolates had 1 virulence factor positive. 18 isolates did not have any of the virulence factor to be positive.

TABLE -9 VIRULENCE FACTORS OF KLEBSIELLA PNEUMONIAE (n=19)

Tests for Virulence	Virulent Isolates
Capsule	19(100%)
Biofilm producers	3(15.78%)

Out of the 19 Klebsiella pneumoniae isolates, capsule was present in 19(100%) and 3(15.78%) were biofilm producers.

TABLE -10 VIRULENCE FACTORS OF PROTEUS MIRABILIS (n=2)

Tests for virulence	Virulent Isolates
Hemolysin	2
Biofilm producers	1
Serum resistance	1
Mannose resistant haemagglutination	1

Out of the 2 Proteus mirabilis isolates, hemolysin was positive in 2. Biofilm producer, serum resistance and mannose resistant hemagglutination was positive in 1.

TABLE -11 VIRULENCE FACTORS OF PSEUDOMONAS AERUGINOSA

(n=3)

Tests for virulence	Virulent Isolates
Pyocyanin	3
β-Hemolysin	2
Biofilm Producers	2

Out of the 3 Pseudomonas aeruginosa isolates, 3 were pyocyanin producers and 2 were biofilm producers and β-hemolysin positive.

TABLE -12 VIRULENCE FACTORS OF STAPHYLOCOCCUS AUREUS

(n=21)

Tests for Virulence	Virulent Isolates
Slide coagulase test	21(100%)
Tube Coagulase test	21(100%)
DNase test	21(100%)
Urease	21(100%)
Phosphatase test	20(95.23%)
Hemolysin	12(57.14%)
Biofilm producers	8(38.09%)

Out of 21 isolates of Staphylococcus aureus, Slide coagulase, Tube coagulase, urease and DNase were positive in 21(100%) followed by phosphatase test 20(95.23%), Hemolysin 12(57.14%) and Biofilm producers were 8(38.09%). The p-value for hemolysin (p=0.007), biofilm (p=0.036), tube coagulase (p=<0.001) and phosphatase (p=0.002) were statistically significant.

TABLE -13 VIRULENCE FACTORS OF ENTEROCOCCUS FAECALIS

(n=9)

Tests for Virulence	Virulent Isolates
β-Hemolysin	4(44.44%)
Gelatinase test	2(22.22%)
Biofilm	2(22.22%)

Out of the 9 Enterococcus faecalis isolates, 4(44.44%) were β-hemolysin positive, 2(22.22%) were gelatinase and biofilm producers.

TABLE – 14 ANTIBIOTIC SENSITIVITY PATTERN OF GRAM NEGATIVE ISOLATES (n=78)

Name of the Drug	Escherichia coli (n=54)	Klebsiella pneumoniae (n=19)	Proteus mirabilis (n=2)	Pseudomonas aeruginosa (n=3)
Amoxycillin	24(44.4)%	7(36.8%)	0	Not tested
Amoxycillin/ Clavulanic acid	46(85.2%)	9(47.4%)	1(50%)	2(66.7%)
Cotrimoxazole	39(72.2%)	11(57.9%)	1(50%)	2(66.7%)
Cephalexin	32(59.3%)	7(36.8%)	0	Not tested
Cefotaxime	50(92.6%)	17(89.5%)	2(100%)	Not tested
Ceftazidime	50(92.6%)	17(89.5%)	2(100%)	3(100%)
Gentamicin	35(64.8%)	10(52.6%)	1(50%)	1(33.3%)
Amikacin	42(77.8%)	15(78.9%)	1(50%)	1(33.3%)
Norfloxacin	30(55.6%)	10(52.6%)	1(50%)	1(33.3%)
Ofloxacin	45(83.3%)	13(68.4%)	1(50%)	2(66.7%)
Nitrofurantoin	48(88.9%)	15(78.9%)	0	3(100%)
Piperacillin/ Tazobactum	54(100%)	19(100%)	2(100%)	3(100%)
Imipenem	54(100%)	19(100%)	2(100%)	3(100%)

All the Gram negative bacilli were 100% sensitive to Piperacillin/Tazobactum and Imipenem. Nitrofurantoin showed 48(88.9%) and 15(79%) sensitivity to Escherichia coli and Klebsiella pneumoniae. Amoxycillin showed 24(44.4%) sensitivity to Escherichia coli and 7(36.8%) to Klebsiella pneumoniae. Cefotaxime and ceftazidime showed 92.6% and 89.5% sensitivity to Escherichia coli and Klebsiella pneumoniae.

CHART - VIII

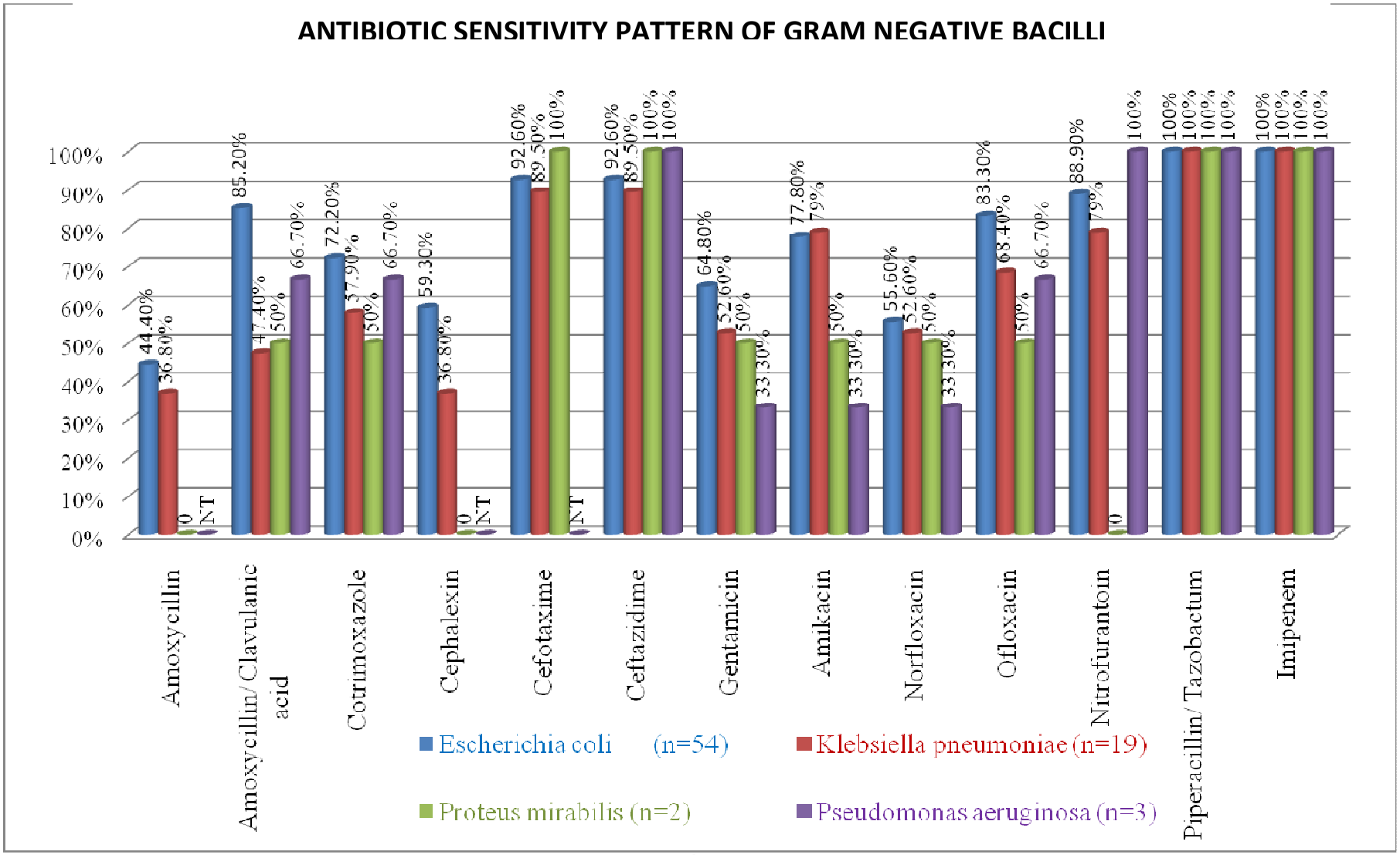

ANTIBIOTIC SENSITIVITY PATTERN OF GRAM NEGATIVE BACILLI
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
44.40%
36.80%
0
NT
85.20%
47.40%
50%
66.70%
72.20%
57.90%
50%
66.70%
59.30%
36.80%
0
NT
92.60%
89.50%
100%
NT
92.60%
89.50%
100%
100%
64.80%
52.60%
50%
33.30%
77.80%
79%
50%
33.30%
55.60%
52.60%
50%
33.30%
83.30%
68.40%
50%
66.70%
88.90%
79%
0
100%
100%
100%
100%
100%
100%
100%
100%
Amoxycillin
Amoxycillin/ Clavulanic acid
Cotrimoxazole
Cephalexin
Cefotaxime
Ceftazidime
Gentamicin
Amikacin
Norfloxacin
Ofloxacin
Nitrofurantoin
Piperacillin/ Tazobactum
Imipenem
Escherichia coli (n=54)
Klebsiella pneumoniae (n=19)
Proteus mirabilis (n=2)
Pseudomonas aeruginosa (n=3)

TABLE – 15 ANTIBIOTIC SENSITIVITY PATTERN OF GRAM POSITIVE ORGANISMS (n=40)

Name of the Drug	Staphylococcus aureus (n=21)	Staphylococcus saprophyticus (n=10)	Enterococcus faecalis (n=9)
Amoxycillin	8(38.09%)	5(50%)	3(33.33%)
Amoxycillin/clavulanic acid	19(90.47%)	10(100%)	6(66.66%)
Cotrimoxazole	10(47.62%)	10(100%)	5(55.55%)
Cephalexin	9(42.85%)	7(70%)	Not tested
Cefotaxime	15(71.42%)	8(80%)	Not tested
Gentamicin	10(47.62%)	6(60%)	5(55.55%)
Amikacin	15(71.42%)	8(80%)	6(66.66%)
Norfloxacin	9(42.85%)	6(60%)	6(66.66%)
Ofloxacin	17(80.95%)	9(90%)	8(88.88%)
Nitrofurantoin	16(76.19%)	8(80%)	7(77.77%)
Vancomycin	21(100%)	10(100%)	9(100%)

All the Gram positive cocci were 100% sensitive to Vancomycin. Staphylococcus aureus and Staphylococcus saprophyticus showed 79.19% and 80% sensitivity to Nitrofurantoin. Staphylococcus aureus isolates were tested for Methicillin resistant Staphylococcus aureus using Cefoxitin disk. None of the Staphylococcus aureus isolates were Methicillin resistant Staphylococcus aureus.

CHART - IX

ANTIBIOTIC SENSITIVITY PATTERN OF GRAM POSITIVE ISOLATES

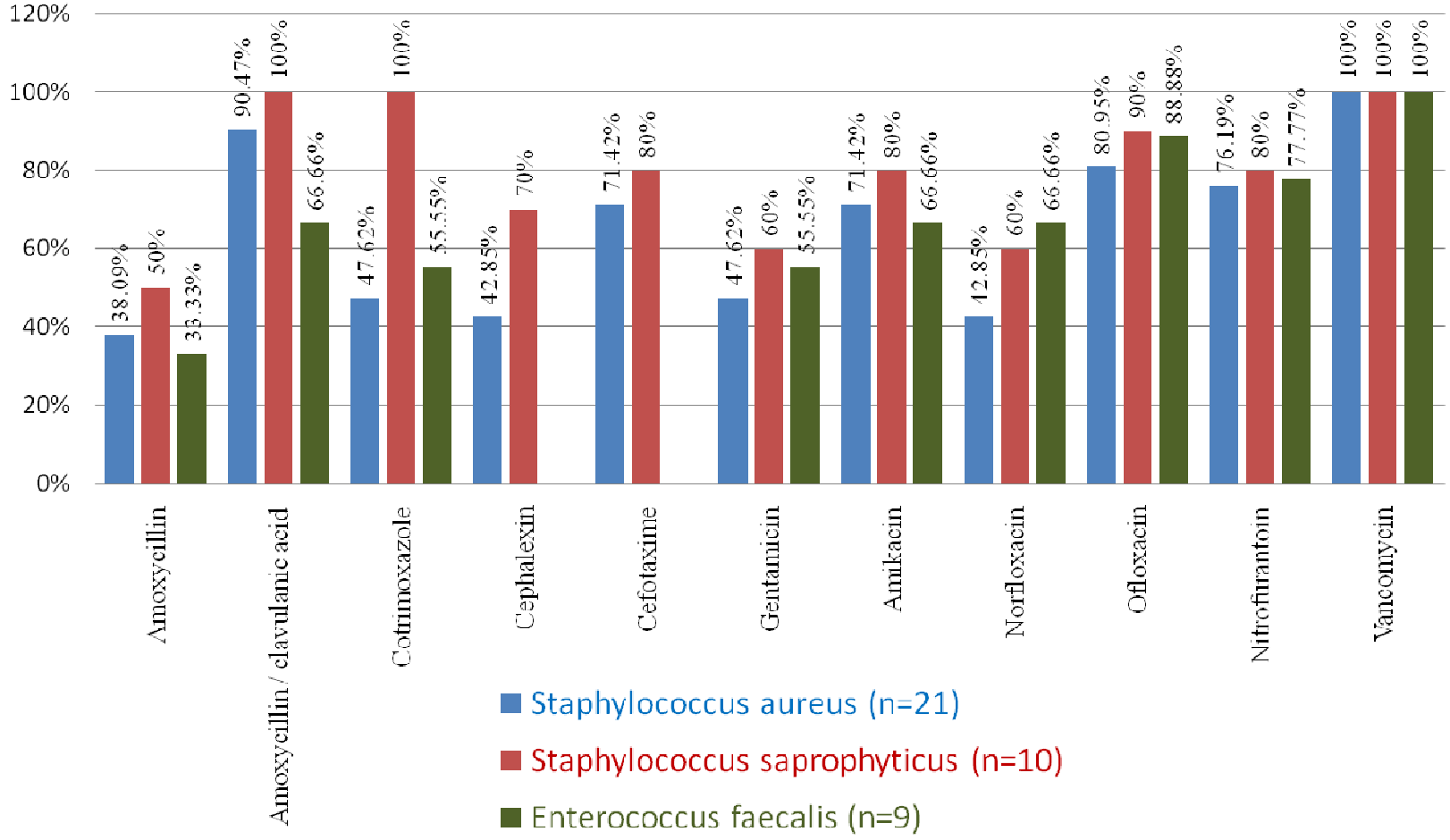


TABLE – 16 SCREENING TEST FOR ESBL DETECTION AMONG THE ENTEROBACTERIACEAE (n=75)

Organism	Total Number of Organisms	Resistant to cefotaxime and ceftazidime
Escherichia coli	54	4(7.4%)
Klebsiella pneumoniae	19	2(10.5%)
Proteus mirabilis	2	0

Out of 75 Enterobacteriaceae isolates, 4(7.4%) Escherichia coli and 2(10.5%) Klebsiella pneumoniae were positive by the screening test. None of Proteus mirabilis isolates were positive in the screening test.

TABLE-17 ESBL PRODUCERS BY PHENOTYPIC CONFIRMATORY TEST (n=75)

Organism	Total Number of Organisms	Phenotypic confirmatory test
Escherichia coli	54	4(7.4%)
Klebsiella pneumoniae	19	2(10.5%)
Proteus mirabilis	2	0

By the phenotypic confirmatory test, 4(7.4%) Escherichia coli and 2(10.5%) Klebsiellae pneumoniae were identified as ESBL producers. None of the Proteus mirabilis isolates were positive for ESBL.

TABLE- 18 MINIMUM INHIBITORY CONCENTRATION OF ESBL POSITIVE ISOLATES USING E-STRIP CONTAINING CEFTAZIDIME AND CEFTAZIDIME/CLAVULANIC ACID (n=6)

Serial number	Name of the Organism	Ceftazidime MIC	Ceftazidime + Clavulanic Acid MIC	Ratio TZ/TZL	Result
1.	Escherichia coli	>32	0.25	128	Positive
2.	Escherichia coli	6	0.125	48	Positive
3.	Escherichia coli	>32	0.25	128	Positive
4.	Escherichia coli	3	0.5	6	Negative
5.	K.pneumoniae	6	0.125	48	Positive
6.	K.pneumoniae	3	0.75	4	Negative

Of the 4 ESBL positive Escherichia coli, 3(75%) and of the 2 ESBL positive Klebsiella pneumoniae 1(50%) showed ≥ 8 times reduction in Minimum Inhibitory Concentration.

Table - 19 GENE IDENTIFICATION IN ESBL POSITIVE ISOLATES FOR TEM, SHV and CTX-M (n=6)

Organism	Total Tested	TEM Positive	SHV Positive	CTX –M Positive
Escherichia coli	4	0	0	2
Klebsiella pneumoniae	2	0	0	1

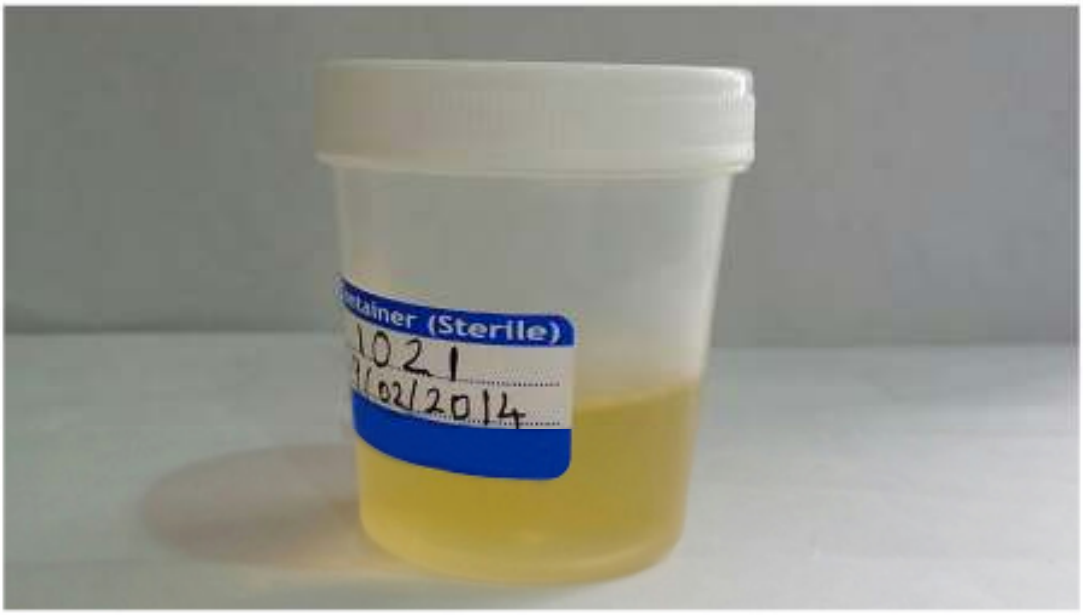
Of the 6 ESBL producers, 3 were positive for CTX-M gene. 2 were Escherichia coli and 1 was Klebsiella pneumoniae. None of the isolates were positive for the TEM and SHV gene.

Table - 20 GENE IDENTIFICATION IN MANNOSE RESISTANT HAEMAGGLUTINATION POSITIVE ISOLATES FOR Pap A GENE BY PCR

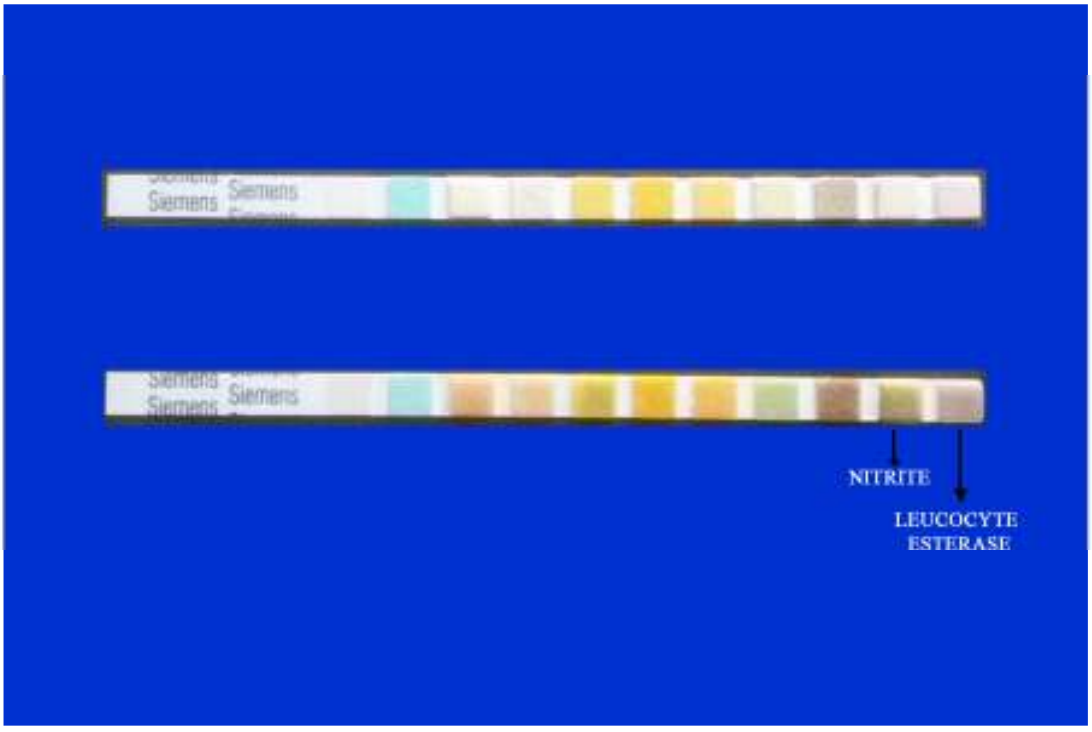
Organism	Mannose Resistant Haemagglutination positive Escherichia coli isolates (P fimbriae)	Pap A GENE %
Escherichia coli	9	9(100%)

Of the 54 Escherichia coli isolates, 9 were positive to mannose resistant hemagglutination test by phenotypic method and they were positive by genotypic method.

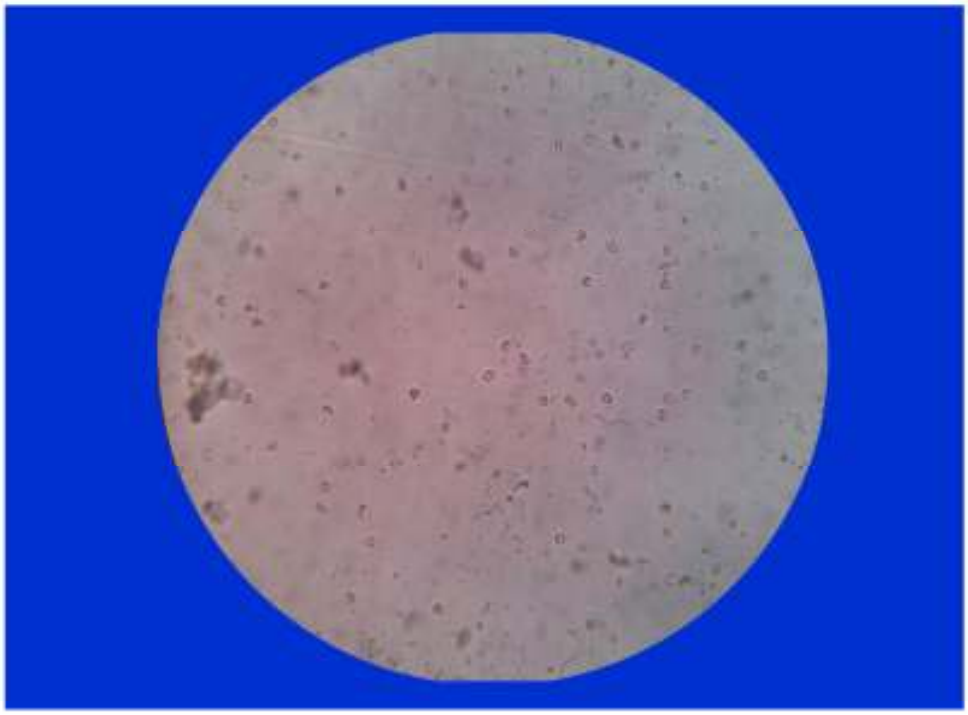
SAMPLE COLLECTION IN STERILE CONTAINER



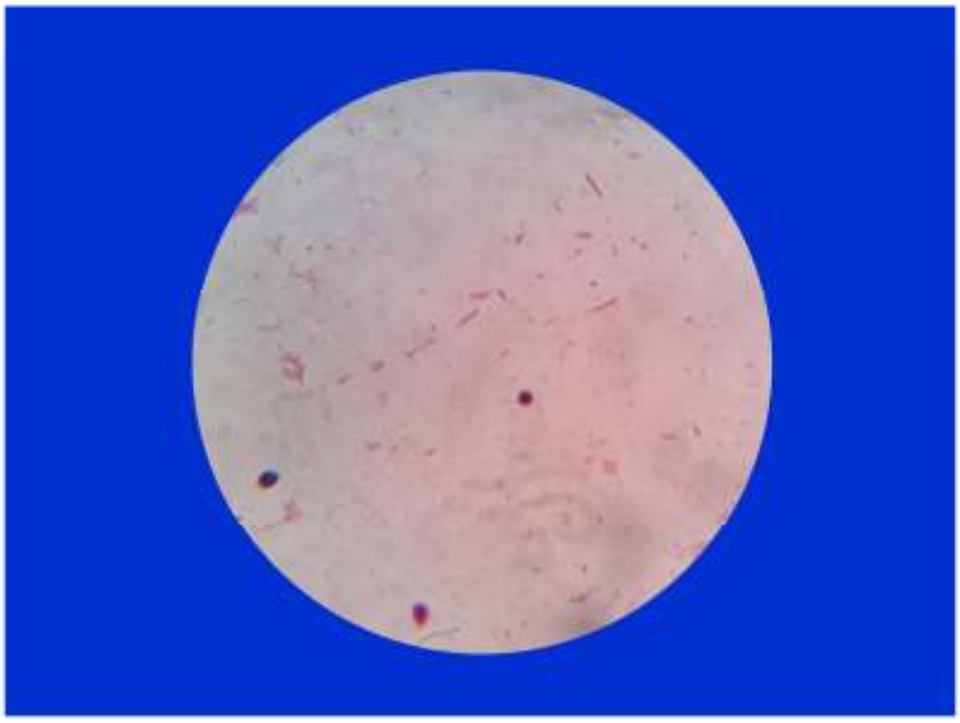
MULTIREAGENT URINE DIPSTICKS



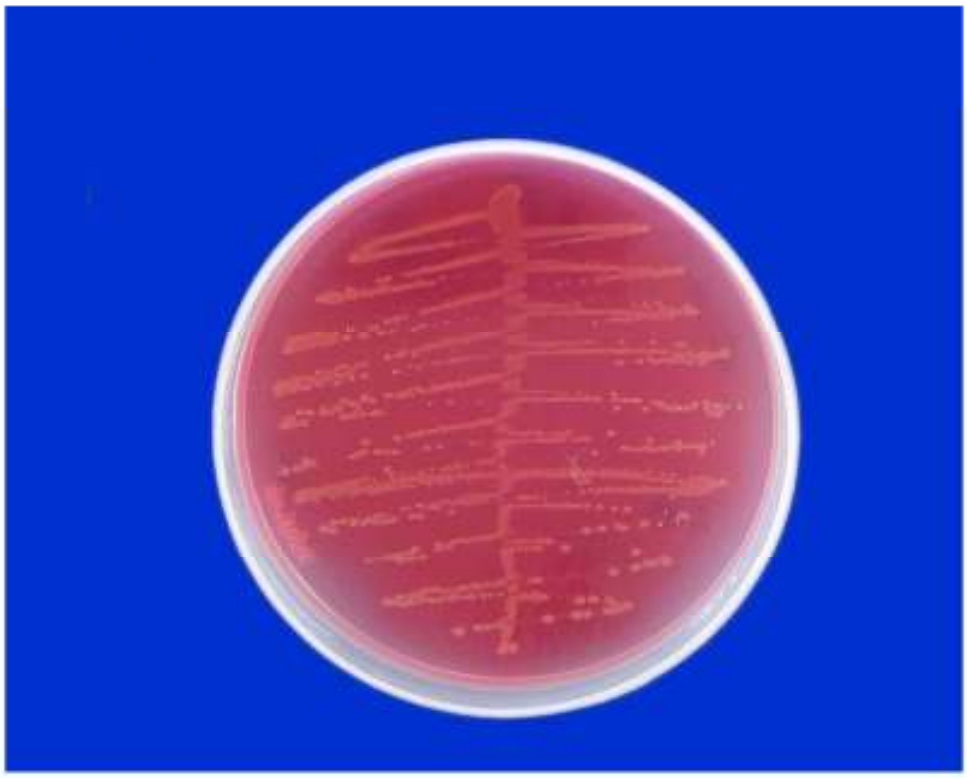
PUS CELLS SEEN IN WET MOUNT



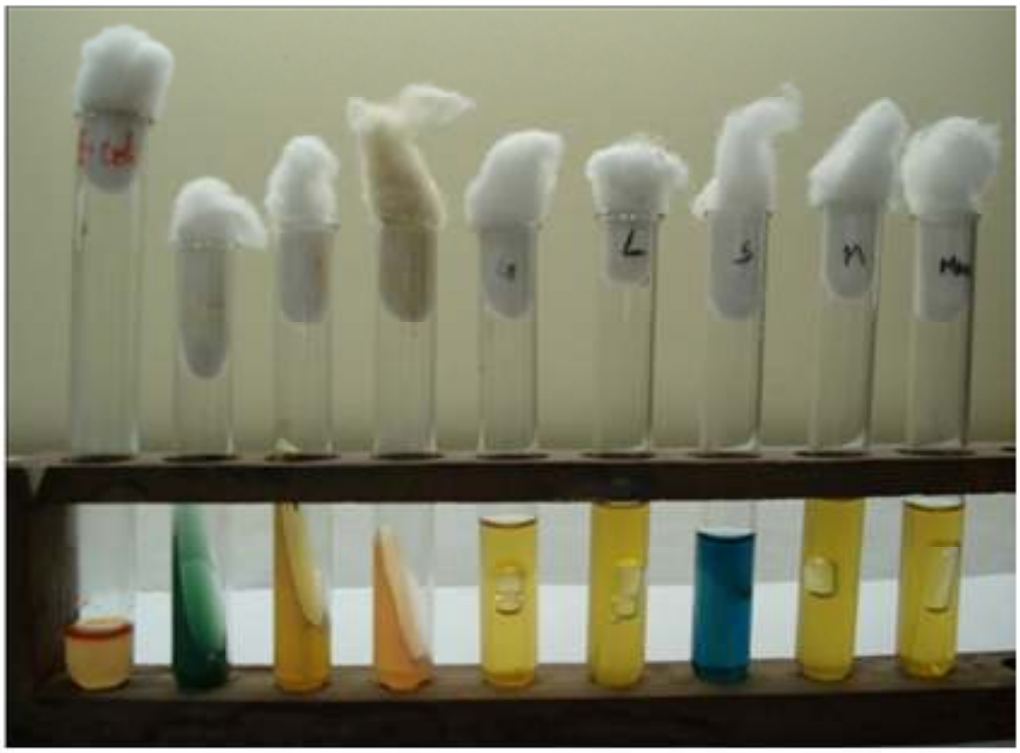
DIRECT GRAM STAIN OF URINE



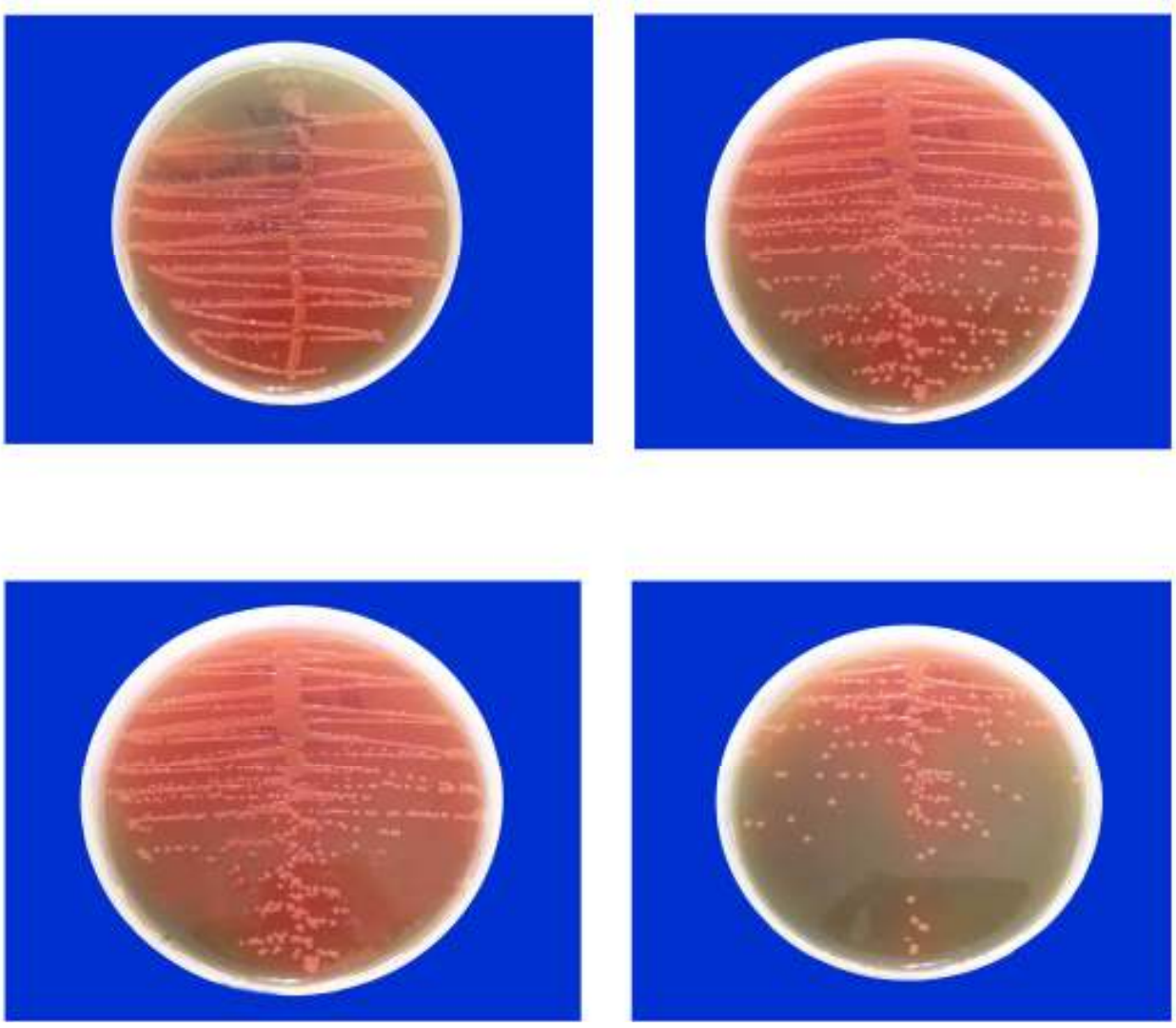
SEMIQUANTITATIVE URINE CULTURE ON MAC CONKEY AGAR



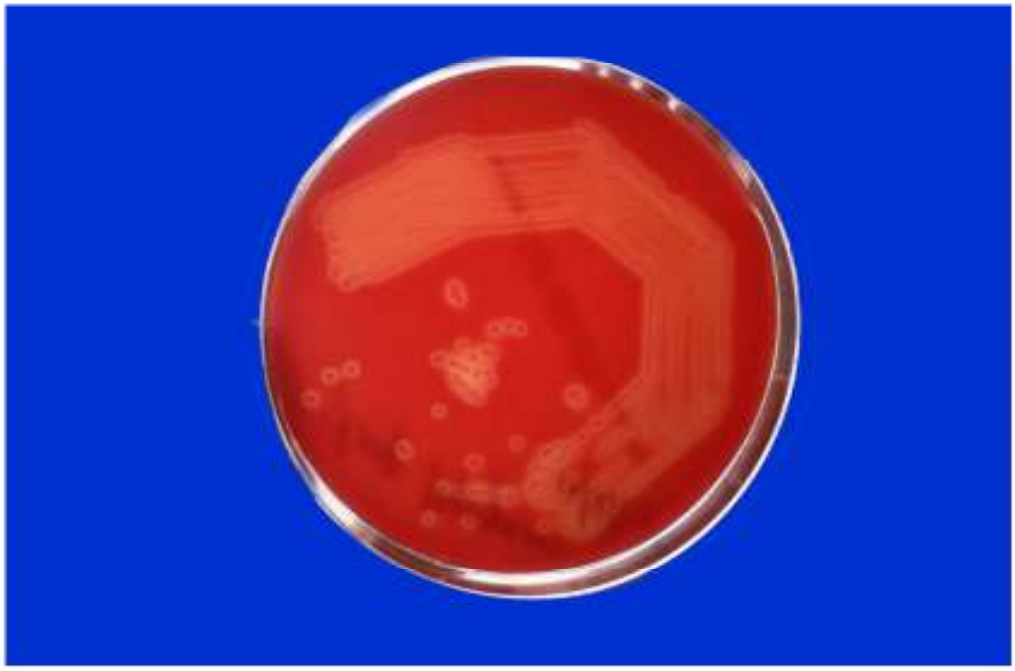
BIOCHEMICAL REACTIONS OF ESCHERICHIA COLI



SEMIQUANTITATIVE URINE CULTURE ON CLED AGAR

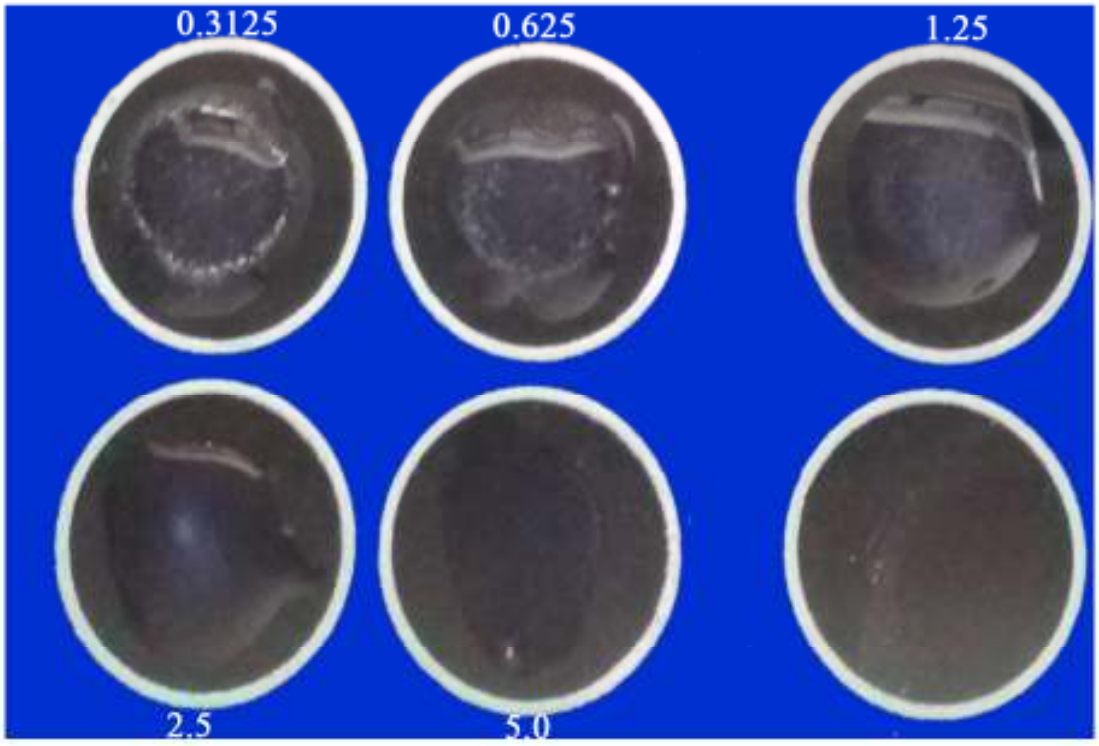


ESCHERICHIA COLI SHOWING HEMOLYSYS



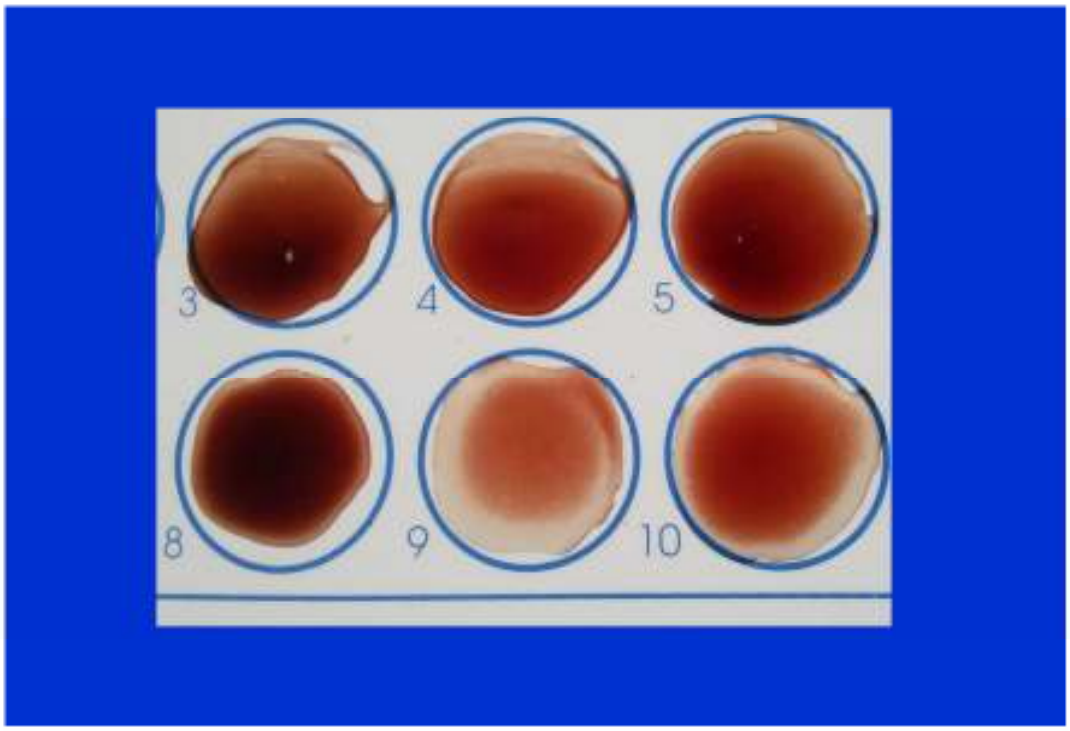
GELATINASE TEST

CELL SURFACE HYDROPHOBOCITY

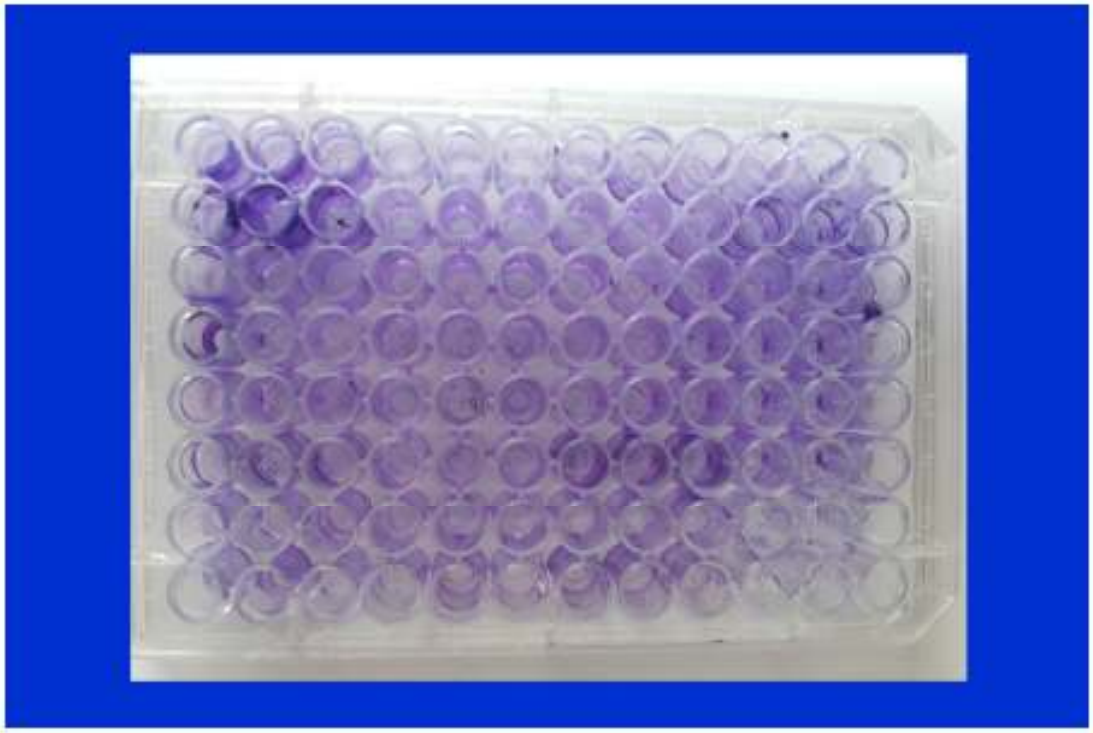


SERUM RESISTANCE

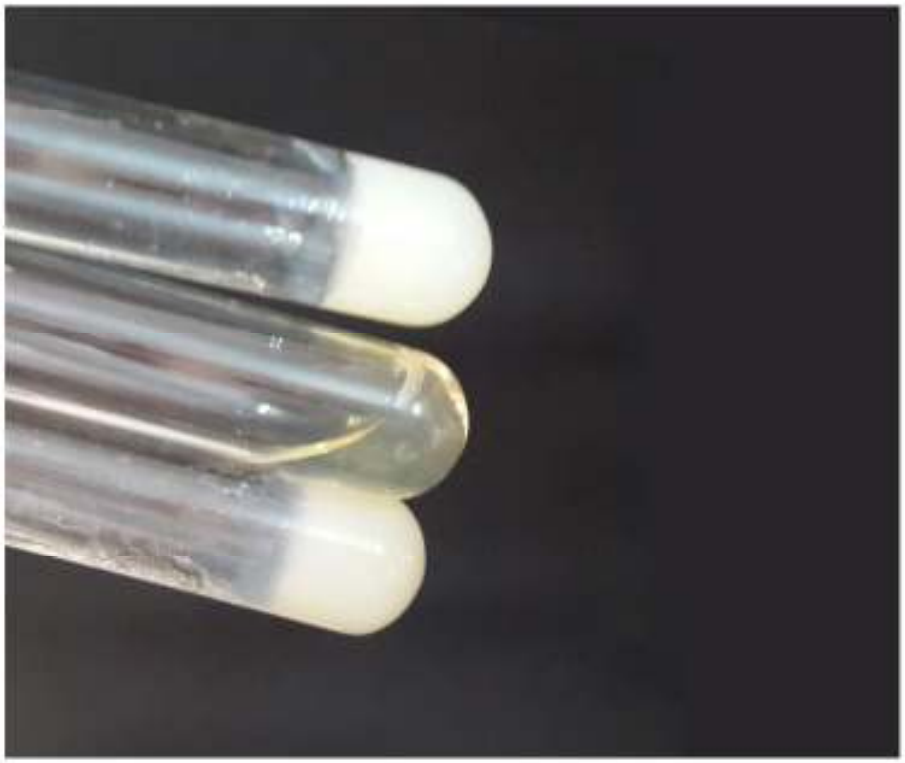
MANNOSE RESISTANT HEAMAGGLUTINATION



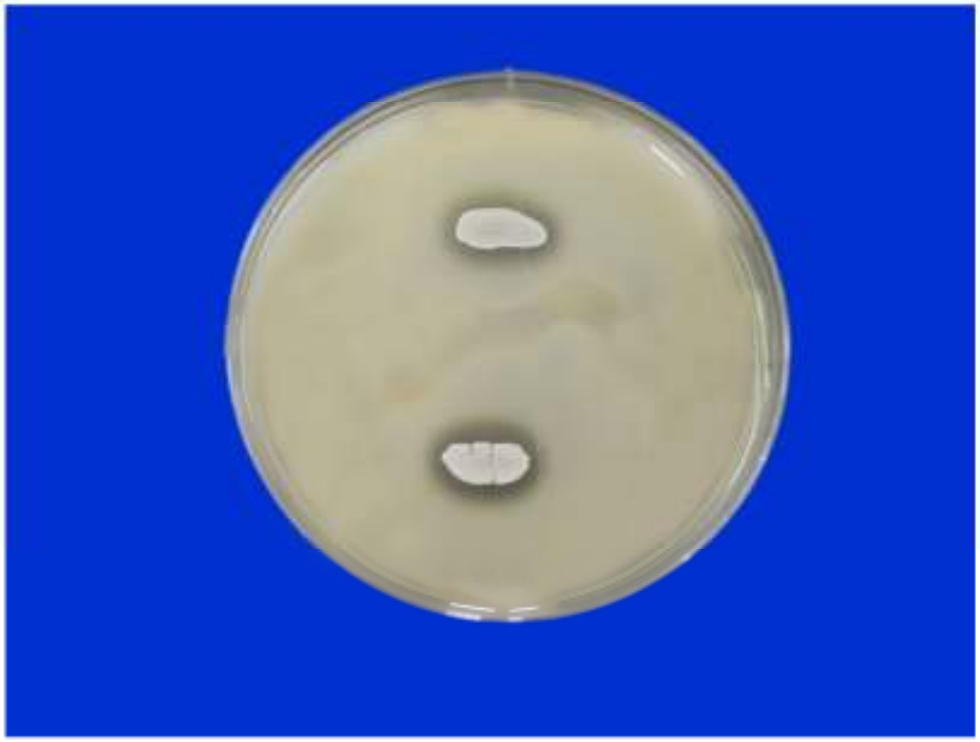
BIOFILM DETECTION BY MICROTITRE PLATE METHOD



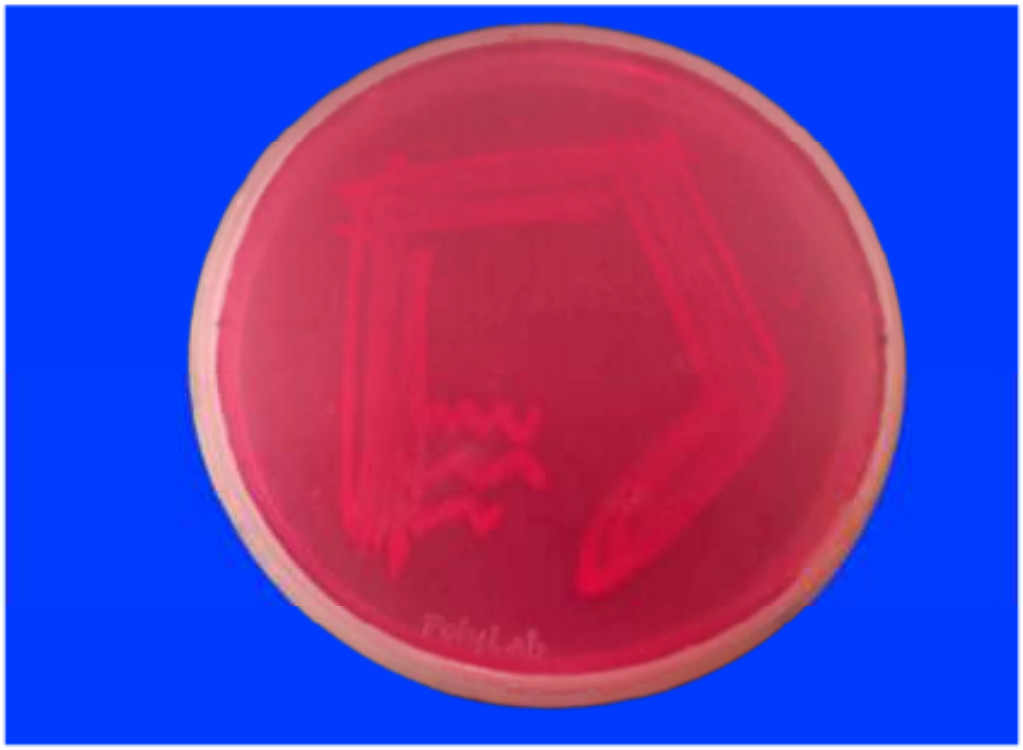
TUBE COAGULASE TEST



DNase TEST



PHOSPHATASE TEST

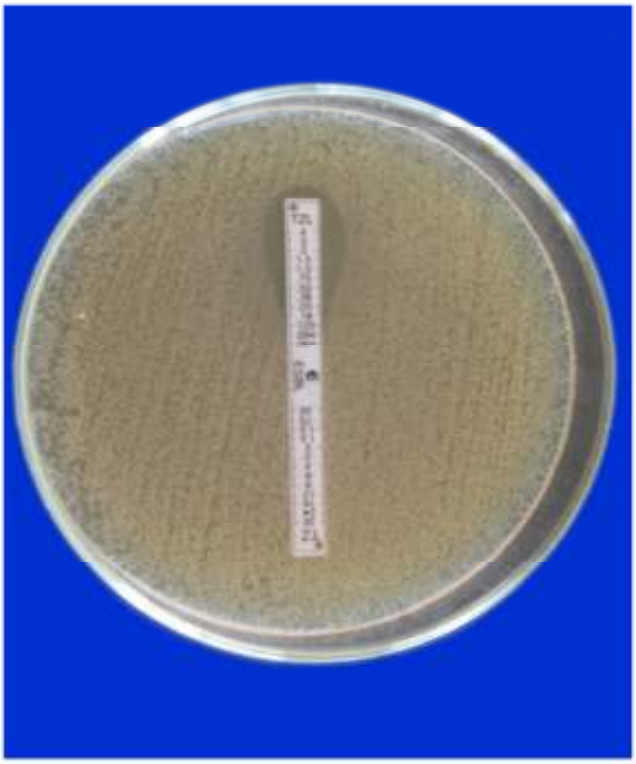


STAPHYLOCOCCUS AUREUS SHOWING HEMOLYSIS

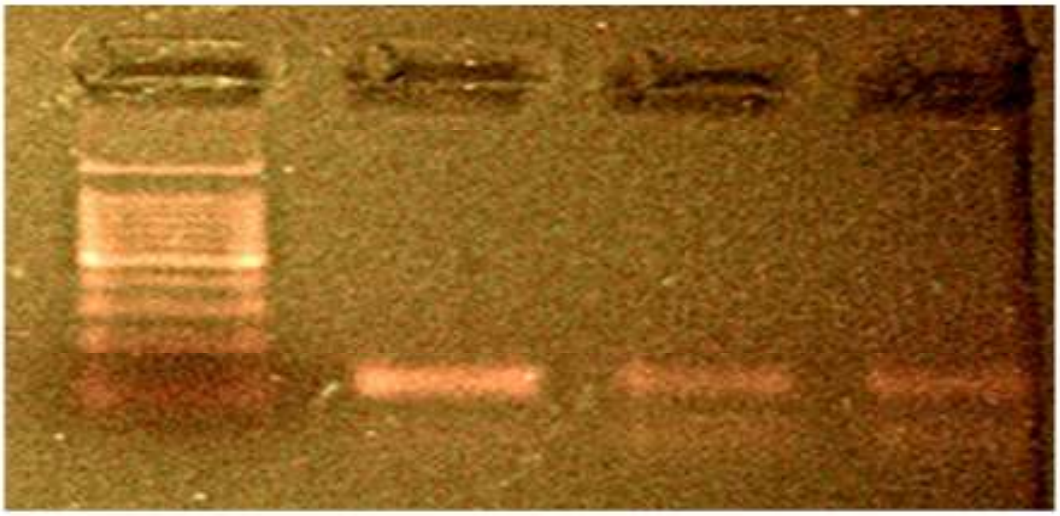
PHENOTYPIC CONFIRMATORY TEST FOR ESBL DETECTION

CAZ = Ceftazidime 30 µg
CAZ+CLAV = Ceftazidime 30 µg+clavulanic acid 10 µg(≥5mm than CAZ)

ESBL DETECTON BY E-STRIP METHOD

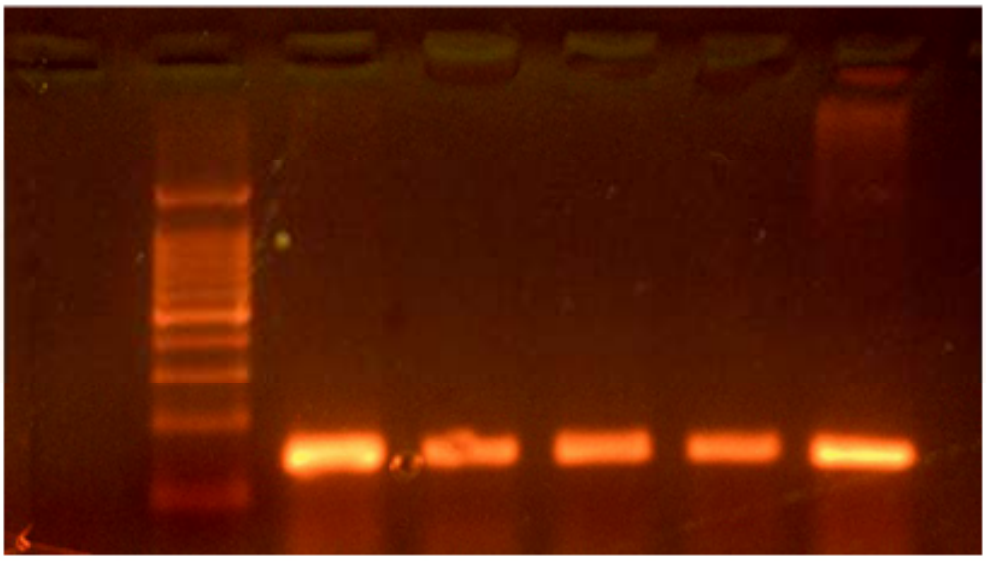


CTX-M gene



Lane One	:	DNA Ladder
Lane Two	:	120 bp
Lane Three	:	120 bp

pap A gene



Lane One	:	Negative control
Lane Two	:	DNA Ladder
Lane Three	:	100 bp
Lane Four	:	100 bp
Lane Five	:	100 bp

DISCUSSION

DISCUSSION

This study aims at determining the common bacterial isolates causing asymptomatic bacteriuria in pregnancy, the virulence factors of various isolates, their antimicrobial susceptibility pattern and the extended spectrum beta lactamase producers. This study was conducted from January 2014 to June 2015 among 1000 antenatal women from 18 to 40 years of age without any symptoms of urinary tract infection. The observations were recorded, analysed and discussed as follows.

As per table – 1, Number of culture positive urine samples were 118(11.8%). This was coinciding with the studies done by Sanyogita Jain et al[9] 11%, Rajashekar et al[12] 11%, Anandhi Kasinathan et al[28] 12.6%, whereas Sudha Birader Kerure et al[11] had 9%, SV Lavanya et al[4] 8.4%, Kheya mukherjee et al[37] 8.4%, R Sujatha et al[6] 7.3% and Gayathree L et al[61] 6.2%,

As per table – 2, the age groups included were from 18-40 years. Pregnant women in the age group of 21-30 years were more culture positive 78(66.10%). This was in accordance to the study done by R Sujatha et al[6] 16(72.72%) and Chandel lata R et al[10] 91.2% whereas in a study done by S V Lavanya et al[4] the culture positivity was significant in the < 20 years age group 71.42%, while in a study done by Jeyaseelan et al[7] it was significant in the > 30 years age group 53.5%. The reason may be due to that, women of this age group are mostly multiparous, and multiparity is a risk factor for getting asymptomatic bacteriuria in pregnancy.

As per table – 3, Urine samples were collected upto gravida 4. The culture positives in asymptomatic bacteriuria were higher in the primigravida 58(49.2%). These findings were in accordance with the observations done by Chandel Lata R et al[10] 52.9%, Kheya Mukherjee et al[37] 11(52.38%), Sudha Birader Kerure et al[11] 56%, Rajashekar et al[12] 59%, S V Lavanya et al[4] 28(66.6%). This was in contrast to the study done by R Sujatha et al[6] for whom the culture positives were significant in multigravida 51.1%. The higher incidence in primigravida may be because physiological and anatomical changes are more marked in primigravida.

As per table – 4, all the three trimesters were included in the study. The culture positives in asymptomatic bacteriuria were significant in the first trimester 38.98% in this study. This was similar to the study done by by R Sujatha et al[6] 45.45%. This was in contrast to the study done by Kheya Mukherjee et al[37] for whom the culture positives were more in the second trimester 42.86%, and in a study done by Gayathree et al[61] it was in the third trimester 61.77%. Hormonal changes occurring prior to the anatomical changes may be the reason behind.

As per table – 5, Pus cells were present in 67(56.7%) by wet mount method. This was in accordance with the study done by Kheya mukherjee et al[37]and J Jayalakshmi et al[17], whereas in a study done by Sushama S et al[40] it was 72.41%.

Direct Gram stain smear positive for organisms were 93(78.81%). This was similar to the studies done by J Jayalakshmi et al[17] and Kheya mukherjee et al[37] whereas in a study done by Gayathree et al[61] it is 90.32%.

Among the screening tests, leukocyte esterase test was positive in 71(60.16%) by dipstick in this study. This was similar to the study done by Kheya mukherjee et al[37], J Jayalakhmi et al[17] and Gayathree et al[61]. This was in contrast to the study done by A Titoria et al[60] (27.5%).

Nitrite test was positive in 89(75.4%) by dipstick method. This was similar to the studies done by J Jayalakshmi et al[17], Kheya Mukherjee et al[37] and Gayathree et al[61].

As per table 6, Gram negative bacilli 78(66.10%) were the predominant organisms isolated than the Gram positive cocci 40(33.90%). This finding was similar with the findings reported by Sanyogita Jain et al[9], Kheya mukherjee et al[37], R Sujatha et al[6], T. Jeyaseelan Senthinath et al[7] and Chandel Lata R et al[10].

As per table 7, The predominant organism isolated was Escherichia coli 54(45.76%), which was in accordance with the study done by Gayathree L et al[61], SV Lavanya et al[4], Ananthi Kasinathan et al[28], Sudha Biradar Kerure et al[11], Chandel Lata et al[10], R.Sujata et al[6], Sanyogita Jain et al[9], A.Titoria et al[60], Kheya Mukherjee et al[37] and Imade et al[85]. In a study done by Ajayi AB et al[87] in Nigeria, Staphylococcus aureus was the most common organism isolated which was 70%, a contrast observation.

Next to Escherichia coli, the organism most commonly isolated was Staphylococcus aureus 21(17.80%). Chandel Lata et al[10], Imade et al[85], Sudha Biradar

Kerure et al[11] and Rajashekar et al[12] also reported Staphylococcus aureus next to Escherichia coli which was similar to this study.

Next to Staphylococcus aureus, the organisms isolated were Klebsiella pneumoniae 19(16.10%), Staphylococcus saprophyticus 10(8.45%), Enterococcus faecalis 9(7.63%), Pseudomonas aeruginosa 3(2.54%) and Proteus mirabilis 2(1.69%).

As per table – 8A, among the virulence factors of Escherichia coli, hemolysin was the predominant virulence factor 18(33.33%). This was similar to the study done by R Raksha et al[2]. Colonisation with haemolytic strains of Escherichia coli are more prone to develop into urinary tract infections.

Shruthi N et al[1] and Rebecca Naveen et al[34], showed mannose resistant haemagglutination as the predominant virulence factor and in studies done by S Sharma et al[63] and Rohini P et al[84], serum resistance was the predominant virulence factor. The other virulence tests that were positive were cell surface hydrophobicity and serum resistance 11(20.37%), gelatinase 10(18.51%), mannose resistant hemagglutination 9(16.66%) and biofilm producers were 6(11.11%). Saroj Golia et al[65] had no biofilm positive isolates among the asymptomatic bacteriuria patients and this is a contrast study.

As per table- 8B, Out of the 54 Escherichia coli isolates, 5 isolates had 3 virulence factors positive, 19 isolates had 2 virulence factors positive and 12 isolates had 1 virulence factor positive. 18 isolates did not have any of the virulence factors to be positive. Similarly, Shruthi N et al[1]had three virulence factors positive in her study.

In a study done by S Sharma et al[63], expression of multiple virulence factors by extra intestinal Escherichia coli was revealed.

As per table - 9, among the virulence factors of Klebsiella pneumoniae, capsule was present in 19(100%) and the biofilm producers were 3(15.78%). Biofilm was positive in Klebsiella pneumoniae in a study done by Hassan et al[86].

As per Table – 10, among the virulence factors of Proteus mirabilis, hemolysin was positive in 2. Biofilm producer, mannose resistant hemagglutination and serum resistance was positive in 1. This was similar to the studies done by M Mishra et al[68] and R Wasfi et al[33].

As per table – 11, among the virulence factors of Pseudomonas aeruginosa, biofilm producers and β-hemolysin was positive in 2 and pyocyanin was positive in 3. Pyocyanin production was considered as a virulence factor in the studies done by P Gupta et al[70] and Lars E.P. Dietrich et al[69].

As per table - 12, among the virulence factors of Staphylococcus aureus, slide coagulase, tube coagulase, urease and DNase were positive in 21(100%), phosphatase was positive in 20(95.23%), biofilm producers were 8(40%), hemolysin was positive in 12(60%).

As per table – 13, among the virulence factors of Enterococcus faecalis gelatinase was positive in 2(22.22%), hemolysin was positive in 4(44.44%) and biofilm producers were 2(22.22%). This was similar to the study done by Praharaj Ira et al[71]. Biofilm shows increased resistance to broad spectrum antibiotics. So biofilm adds to the virulence profile of microorganisms.

As per table – 14, All the Gram negative bacilli were 100% sensitive to Piperacillin/Tazobactum and Imipenem. This was similar to the study done by Girish Babu et al[82]. Cefotaxime and ceftazidime showed 50(92.6%) sensitivity to Escherichia coli. Nitrofurantoin showed 48(88.9%) sensitivity to Escherichia coli and 15(79%) sensitivity to Klebsiella pneumoniae. Amoxycillin showed 24(44.4%) sensitivity to Escherichia coli and 7(36.8%) sensitivity to Klebsiella pneumoniae.

In a study done by Girish Babu et al[82], Nitrofurantoin showed increased sensitivity compared to Cefotaxime and Ceftazidime whereas in this study cefotaxime and ceftazidime were more sensitive compared to nitrofurantoin for the Escherichia coli and Klebsiella pneumoniae isolates.

As per table – 15, All the Gram positive cocci were 100% sensitive to Vancomycin. Staphylococcus aureus and Staphylococcus saprophyticus showed 79.19% and 80% sensitivity to Nitrofurantoin. Amoxycillin and amoxycillin/clavulanic acid showed 8(38.09%) and 19(90.47) sensitivity to Staphylococcus aureus. Amoxycillin showed 5(50%) sensitivity to Staphylococcus saprophyticus and 3(33.30%) to Enterococcus faecalis. This was similar to a study done by Kheya Mukherjee et al[37].

As per table – 16, ESBLs were screened by observing resistance to third generation cephalosporins, cefotaxime and ceftazidime. Out of 54 Escherichia coli isolates, 4 were positive and out of 19 Klebsiella pneumoniae isolates, 2 were positive. None of the Proteus mirabilis isolates were positive for ESBL.

As per table – 17, Escherichia coli and Klebsiella pneumoniae isolates which were resistant to 3rd generation cephalosporins were further confirmed by the phenotypic confirmatory test. Among the 6 ESBL producers, 4 were Escherichia coli and two were Klebsiella pneumoniae isolates. In a study done by R Sujatha et al[6], out of the 17 Escherichia coli isolates, 1 was an ESBL producer.

As per table – 18, Minimum inhibitory concentration (MIC) was done by the E - strip using ceftazidime and ceftazidime/clavulanic acid. 3(75%) were positive for Escherichia coli and 1(50%) was positive for Klebsiella pneumoniae. A ceftazidime: ceftazidime/clavulanic acid MIC ratio of ≥ 8 was considered as a positive result for ESBL production.

As per table – 19, By polymerase chain reaction, 2 out of 4 Escherichia coli and 1 out of 2 Klebsiella pneumoniae were confirmed for CTX- M gene and none were positive for the the TEM and SHV genes.

As per table – 20, by polymerase chain reaction, 9(100%) which were positive for mannose resistant haemagglutination by phenotypic method were positive for the pap A gene. The pap A gene was positive in the following studies done by AV Shetty et al[16], Francisco Toval et al[66], J Agarwal et al[83], Jean – Philippe Lavigne et al[67], and Akira Takahashi et al[36].

As asymptomatic bacteriuria is related to the complications in pregnancy, it is therefore imperative that pregnant women be tested for bacteriuria, periodically in every trimester of the gestational period. Urine culture tests should be done routinely

for all the pregnant women to find out asymptomatic bacteriuria, and every positive case should be managed with appropriate antibiotics, to prevent any complication related to pregnancy. In view of the changing patterns of bacterial resistance to common drugs, the administration of antibiotics must be done cautiously to prevent the emergence of bacterial resistance in the hospital and the community.

SUMMARY

SUMMARY

Mid stream clean catch urine specimens were collected from one thousand antenatal women without any symptoms of urinary tract infection who attended Obstetrics and Gynaecology out patient department at Government Kilpauk Medical College Hospital, Chennai from January 2014 to June 2015. Urine samples were collected from pregnant women of different age groups, gravida and trimesters.

The culture positives with asymptomatic bacteriuria in pregnancy were 11.8%. The culture positives were more in the 21-30 years age group, primigravida and in the first trimester. Gram negative bacilli were 78(66.10%) the predominant bacteria isolated in women with asymptomatic bacteriuria in antenatal women.

Escherichia coli was the major isolate constituting 54(45.76%) followed by Staphylococcus aureus 21(17.8%).

Among the virulence factors of Escherichia coli, hemolysin was the major virulence factor constituting 18(33.33%) followed by cell surface hydrophobicity, serum resistance, gelatinase, mannose resistant hemagglutination and biofilm production.

Among the virulence factors of Staphylococcus aureus, slide, tube coagulase, urease and DNase were positive in 21(100%) followed by Phosphatase, Hemolysin and biofilm production.

Among the virulence factors of Klebsiella pneumoniae, capsule was present in 19(100%) and 3(15.78%) were biofilm producers.

Among the virulence factors of Enterococcus faecalis, hemolysin was positive in 4(44.44%), gelatinase and biofilm producers were 2(22.22%).

Among the virulence factors of Pseudomonas aeruginosa, pyocyanin was positive in 3, hemolysin and biofilm producers were 2.

Among the virulence factors of Proteus mirabilis, hemolysin was positive in 2, biofilm, serum resistance and mannose resistant hemagglutination were positive in 1.

In the present study, antibiotic sensitivity of all Enterobacteriaceae showed 100% sensitivity to imipenem and piperacillin/tazobactam. Pseudomonas aeruginosa showed 3(100%) sensitivity to piperacillin /Tazobactam and imipenem. Escherichia coli 48(88.9%), Klebsiella pneumoniae 15(79%), Pseudomonas aeruginosa 3(100%) and Proteus mirabilis 0% showed sensitivity to nitrofurantoin. Amoxycilllin showed less than 45% sensitivity to all the Gram negative isolates. Cefotaxime and ceftazidime showed 92.6% and 89.5% sensitivity to Escherichia coli and Klebsiella pneumoniae

Antibiotic sensitivity testing of Staphylococcus aureus showed 100% sensitivity to vancomycin. Among 21 Staphylococcus aureus, 17(80.95%) were sensitive to amoxicillin-clavulanic acid, 16(76.19%) to nitrofurantoin and 9(42.85%) to cephalexin. Enterococcus faecalis 9(100%) and Staphylococcus saprophyticus 10(100%) were sensitive to vancomycin. None of the Staphylococcus aureus isolates were Methicillin Resistant Staphylococcus aureus.

ESBLs were screened by detecting resistance to third generation cephalosporins, cefotaxime and ceftazidime. Out of 75 Gram negative bacilli 6(8%)

were ESBL producers. Out of 54 Escherichia coli, 4(7.4%) and out of 19 Klebsiella pneumoniae, 2(10.5%) were ESBL producers. Proteus mirabilis showed 100% sensitivity to both the cephalosporins.

4 Escherichia coli and 2 Klebsiella pneumoniae were positive by the phenotypic confirmatory test. Minimum inhibitory concentration (MIC) was done with E-strip containing ceftazidime and ceftazidime with clavulanic acid for the ESBL producers. Of the 4 ESBL positive Escherichia coli, 3(75%) were positive and of the 2 ESBL positive Klebsiella pneumoniae 1(50%) was positive showing a ceftazidime and ceftazidime/clavulanate MIC ratio of ≥ 8.

6 ESBL producing Enterobacteriaceae which were phenotypically confirmed as ESBL positives were subjected to genotypic test by Polymerase Chain Reaction (PCR). Three genes such as TEM, SHV and CTX-M associated with ESBL production were studied using the relevant primers for the corresponding genes. CTX-M was detected in 3(50%). 9 Mannose resistant haemagglutination positive Escherichia coli isolates were positive for the pap A gene.

In the present study, all the 21 Staphylococcus aureus isolates were Methicillin sensitive Staphylococcus aureus and none were found to be Methicillin resistant Staphylococcus aureus.

CONCLUSION

CONCLUSION

- The present study showed that Gram negative bacilli 78(66.10%) were the predominant bacteria isolated in asymptomatic bacteriuria in antenatal women.
- Among the Gram negative bacilli, Escherichia coli 54(45.76%) was found to be the most common bacterial pathogen isolated and Staphylococcus aureus was the most common Gram positive organism isolated followed by Klebsiella pneumoniae.
- Among the Escherichia coli isolates, hemolysin was the major virulence factor constituting 18(33.33%), followed by cell surface hydrophobicity, serum resistance, gelatinase, mannose resistant hemagglutination and biofilm production.
- Among the virulence factors of Staphylococcus aureus, slide coagulase, tube coagulase, urease and DNase were positive in 21(100%), followed by phosphatase, hemolysin and biofilm.
- Among the Enterobacteriaceae isolates, 6(8%) were found to be ESBL producers and all of them showed 100% sensitivity to imipenem.
- Among Gram positive organisms, Staphylococcus aureus was the predominant isolate.
- Among the Staphylococcus aureus isolates, none were found to be MRSA.
- The present study showed high occurrence of asymptomatic bacteriuria in pregnant women which if not treated, might lead to various maternal and neonatal complications. Urine culture with clean catch mid stream urine is the most sensitive test for its detection preferably in the first trimester. All the

sequelae of asymptomatic bacteriuria during pregnancy could be reduced by antimicrobial treatment early in pregnancy. Hence, screening and management of asymptomatic bacteriuria need to be incorporated as a routine antenatal care for an integrated approach to safe motherhood and newborn health.

ANNEXURES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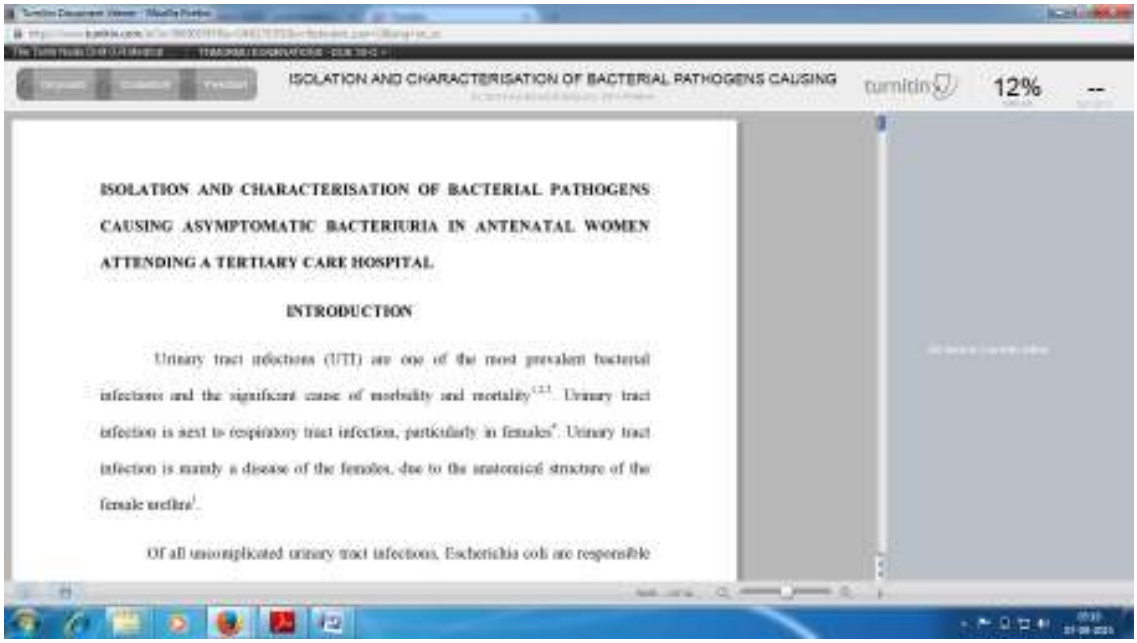
ISOLATION AND CHARACTERISATION OF BACTERIAL PATHOGENS CAUSING
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ISOLATION AND CHARACTERISATION OF BACTERIAL PATHOGENS CAUSING ASYMPTOMATIC BACTERIURIA IN ANTENATAL WOMEN ATTENDING A TERTIARY CARE HOSPITAL
INTRODUCTION
Urinary tract infections (UTI) are one of the most prevalent bacterial infections and the significant cause of morbidity and mortality[1,2,3]. Urinary tract infection is next to respiratory tract infection, particularly in females[4]. Urinary tract infection is mainly a disease of the females, due to the anatomical structure of the female urethra[5].
Of all uncomplicated urinary tract infections, Escherichia coli are responsible

INSTITUTIONAL ETHICAL COMMITTEE
GOVT.KILPAUK MEDICAL COLLEGE,
CHENNAI-10

Ref.No. 19525/ME-1/Ethics/2013 Dt:03.01.2014

CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "A Study on isolation and charaterisation of bacterial pathogens causing asymptomatic bacteriuria in antenatal women attending tertiary care hospital" - For Project work Submitted by Dr.P Prabha, MD (Microbiology), PG Student, KMC, Chennai-10.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

GOVT. KILPAUK MEDICAL COLLEGE * Kilpauk, Chennai-10. * 29 JAN 2014

CHAIRMAN, 29/1/14.
Ethical Committee
Govt.Kilpauk Medical College,
Chennai

PROFORMA

Name : IP no:

Age : Ward:

Sex :

Occupation :

Address :

Gravida :

Para :

LMP :

EDD :

- Underlying illness :
  - Diabetes mellitus.
  - Hypertension
  - Others
- Procedures:
  - Catheterization
- Prior antibiotic therapy :

Laboratory evaluation:

Sample collection Date

Microbiological investigation:

- Macroscopic examination:
- Microscopic examination:

  Gram Stain

  Wet film
- Culture:

  Bacterial culture
- Antimicrobial sensitivity pattern:

சுய ஒப்புதல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு : "ISOLATION AND CHARACTERISATION OF BACTERIAL PATHOGENS CAUSING ASYMPTOMATIC BACTERIURIA IN ANTENATAL WOMEN ATTENDING TERTIARY CARE HOSPITAL"

ஆய்வு செய்யப்படும் இடம்: கீழ்ப்பாக்கம் அரசு மருத்துவக்கல்லூரி மற்றும் மருத்துவமனை, சென்னை -10

பங்கு பெறுபவரின் பெயர்:

பங்கு பெறுபவரின் வயது: பங்கு பெறுபவரின் எண்:

மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டுள்ளது. நான் இவ்வாய்வில் தன்னிச்சையாக பங்கேற்கின்றேன். எந்த காரணத்தினாலோ, எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகிக் கொள்ளலாம் என்று அறிந்து கொண்டேன்.

இந்த ஆய்வு சம்பந்தமாகவோ, இதை சார்ந்து மேலும் மேற்கொள்ளும் போதும் இந்த ஆய்வில் பங்குபெறும் மருத்துவர் என்னுடைய மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளை பார்ப்பதற்கு என் அனுமதி தேவை இல்லை என அறிந்து கொள்கிறேன். இந்த ஆய்வின் மூலம் கிடைக்கும் தகவலையோ, முடிவையோ பயன்படுத்திக் கொள்ள மறுக்கமாட்டேன்.

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக் கொள்கிறேன். இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்று உறுதியளிக்கிறேன்.

பங்கேற்பவரின் கையொப்பம் சாட்சியாளரின் கையோப்பம்

இடம்: இடம்:

தேதி: தேதி:

பங்கேற்பவரின் பெயர் மற்றும் விலாசம்:

ஆய்வாளரின் கையொப்பம்:

இடம்: தேதி:

APPENDIX

GRAM STAIN

Primary stain - Crystal violet 10g

Absolute alcohol - 100ml, Distilled water 1 litre

Grams iodine - iodine 10g

Potassium iodide 20g, Distilled water -1 litre

Acetone

Counter stain-dilute carbolfuchsin

1. Flood the crystal violet for one minute
2. Rinse gently with distilled water
3. Flood the slide with Gram's iodine for one minute
4. Rinse gently with distilled water
5. Decolourise with acetone for only 2-3 seconds.
6. Rinse gently with distilled water to remove excess of decolouriser
7. Flood the slide with dilute carbolfuchsin for one minute
8. Rinse the slide with distilled water, air dry and examine under oil immersion objective

PEPTONE WATER

Peptone	10g
Sodium chloride	5g
Distilled water	1 litre

Dissolve the ingredients in warm water, adjust the pH to 7.4 - 7.5 and filter. Distribute as required and autoclave at 121 degree celsius for 15 minutes.

Mac Conkey agar:-

This is a useful medium for the cultivation of Enterobacteriaceae. It contains a bile salt to inhibit non-intestinal bacteria and lactose with neutral red to distinguish the lactose- fermenting coli forms from the lactose-non-fermenting salmonella and shigella groups. The concentration of sodium taurocholate may be reduced to suit less tolerant organisms. The omission of sodium chloride from the medium prevents the spreading of Proteus colonies.

Peptone	20 g
Sodium taurocholate, commercial	5 g
Water	1 litre
Agar	20 g
Neutral red solution, 2% in 50% ethanol	3.5 ml
Lactose, 10% aqueous solution	100 ml

Dissolve the peptone and taurocholate (bile salt) in the water by heating. Add the agar and dissolve it in the steamer or autoclave. If necessary, clear by filtration. Adjust the pH to 7.5. Add the lactose and the neutral red, which should be well shaken before use, and mix. Heat in the autoclave with 'free steam' (100^{o} C) for 1hour, then at 115^{o} C for 15 min. Pour plates.

NUTRIENT AGAR:-	grams/litre
Peptic digest of animal tissue	5.00
Beef extract	1.50
Yeast extract	5.00
Agar	15.00

Dissolve the contents in water and mix by heating Autoclave at 121^{o} C for 15 minutes. Adjust pH to 7.4 + 0.2. Pour 20-25 ml into 9 cm diameter petridishes to give 4 mm thickness.

BLOOD AGAR:

Sterile sheep blood	50 ml
Peptone	10 g
Beef extract	3g
Sodium chloride	5 g
Distilled water	1000 ml

Autoclave the nutrient agar base at 121^{o} C for 15 minutes and then add blood when the nutrient agar is cooled to 50^{0}C with sterile precautions and distribute in Petri dishes.

MUELLER HINTON AGAR:-

Beef infusion	300 ml
Casein Hydrolysate	17 gm
Starch	1.5 gm
Agar	10 gm
Distilled water	1000 ml

Emulsify the starch in a small amount of cold water, pour into the beef infusion and add the casein-hydrolysate and the agar. Make up the volume to 1000 ml (1 litre) with distilled water. Dissolve the constituents by heating gently at 100^{o} C with agitation.

Adjust the pH to 7.4. Dispense in screw-capped bottles and sterilize by autoclaving at 121 ^{o} C for 20 minutes. 20 to 25 ml of it is poured into petridishes of 9 cm diameter to give a thickness of 4mm.

McFarland's Turbidity Standard for inoculum preparation

A Barium sulphate 0.5 McFarland standards was prepared as follows

1. A 0.5 ml of 0.048mol/L of Barium chloride was added to 99.5 ml of 0.18 mol/L of H2SO4 with constant stirring to maintain a suspension.
2. Correct density of the turbidity standard was verified by using a spectrophotometer. The absorbance of 625nm should be 0.08 to 0.10 for the 0.5 McFarland standards.
3. The Barium sulphate suspension was transferred in 4-6 ml to a screw capped tube of the same size as those used in growing or diluting the bacterial inoculum.
4. These tubes were tightly sealed and stored in the dark at room temperature.
5. The Barium sulphate turbidity standard was vigorously agitated before each use and inspected for a uniform turbid appearance.

Differentiating Characters of Isolates Commonly Observed In Samples of Asymptomatic Bacteriuria in Antenatal Women

Organism	TSI	Citrate	Indole	Oxidase	Catalase	Urease	Glucose	Lactose	Sucrose	Maltose	Mannose	Motility
Escherichia coli	A/A with gas	Not utilised	+	-	+	-	+	+	-	+	+	+
Klebsiella pneumoniae	A/A with gas	utilised	-	-	+	+	+	+	+	+	+	-
Proteus species	K/A with H2S	variable	±	-	+	+	+	-	-	-	-	+
Pseudomonas aeruginosa	K/No change	utilised	-	+	+	-	+	-	-	+	-	+

Note: A/A = Acid slant / Acid butt, + = Positive, - = Negative, v - Variable

Differentiating Characters Of Isolates Commonly Observed In Samples of Asymptomatic Bacteriuria in Antenatal Women

Gram strain	Catalase	Oxidase	Hemolysis	Coagulase	Mannitol	Bile esculin agar	Novobiocin	Isolate
Cocci in cluster	Positive	negative	±	Positive	Positive	Negative	Sensitive	Staphylococcus aureus
Cocci in cluster	Positive	negative	±	Negative	Negative	Negative	Resistant	Staphylococcus saprophyticus
Cocci in pairs	Positive	negative	±	Negative	Negative	Positive	-	Enterococcus species

ZONE SIZE INTERPRETATIVE CHART IN ACCORDING TO NCCLS

Kirby-Bauer Chart

Sl. No.	Drug	Disk Content µg	Resistant mm or less	Intermediate mm	Sensitive mm or more
1	Amoxycillin	10	14 mm	15-16 mm	17 mm
2	Cotrimoxazole	1.25/23.75	10	11-15	16
3	Cephalexin	30	14	15-17	18
4	Ofloxacin	5	12	13-15	16
5	Ciprofloxacin	5	15	16-20	21
6	Gentamicin	10	12	13-14	15
7	Cefotaxime	30	14	15-22	23
8	Ceftazidime	30	14	15-17	18
9	Linezolid	30	-	-	21
10	Amikacin	30	14	15-16	17
11	Imipenem	10	13	15-16	16
12	Piperacillin/tazobactum	100/10	17	18-20	21
13	Vancomycin	30	-	-	15
14	Nitrofurantoin	300	14	15-16	17

ABBREVIATION

ATCC	-	American Type Culture Collection.
CLSI	-	Central Laboratory Standards Institute
CLED	-	Cysteine Lactose Electrolyte Deficient
CFU	-	Colony Forming Units.
ESBL	-	Extended Spectrum Beta Lactamase
E- Test	-	Epsilometer Test
IUGR	-	Intra Uterine Growth Retardation
MHA	-	Mueller Hinton Agar
MIC	-	Minimum Inhibition Concentration
PCR	-	Polymerase Chain Reaction
PBP	-	Pencillin Binding Protein
SHV	-	Sulphy Hydryl Variable
TTC	-	Triphenyl Tetrazolium Chloride Test
TEM	-	Temoniera
UTI	-	Urinary Tract infection
UPEC	-	UroPathogenic Escherichia coli

KEY TO MASTER CHART

OP No	-	Outpatient number
G	-	Gravida
P	-	Para
L	-	Live birth
GA	-	Gross appearance
NG	-	No growth
GNB	-	Gram Negative Bacilli
GPC	-	Gram Positive Cocci
AMX	-	Amoxycillin
AMC	-	Amoxycillin + Clavulanic acid
E	-	Erythromycin
CN	-	Cephalexin
CTX	-	Cefotaxime
CAZ	-	Ceftazidime
AK	-	Amikacin

GEN	-	Gentamicin
OF	-	Ofloxacin
NX	-	Norfloxacin
NIT	-	Nitrofurantoin
COT	-	Cotrimoxazole
PIT	-	Piperacillin+Tazobactum
IMP	-	Imipenem
VAN	-	Vancomycin
CX	-	Cefoxitin
S	-	Sensitive
R	-	Resistant
ESBL	-	Extended Spectrum Beta Lactamases

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Shruthi N, Ravikumar, Ravish kumar. Phenotypic study of virulence factors in Escherichia coli isolated from Antenatal cases, catheterised patients, and faecal flora. J Clin Diagn Res. Dec 2012; 6(10):1699-1703.
2. R Raksha, H Srinivasa, RS Macaden. Occurence and characterisation of uropathogenic Escherichia coli in urinary tract infections. Indian J Med Micribiol. 2003; 21(2):102-107.
3. Balamurugan S, Chaitanya shah, Jayapriya S, Priyadharshini S, Jeya M et al. Reagent strip testing for asymptomatic bacteriuria in pregnant women: A cost effective screening tool in under-resourced settings. J Clin Diagn Res. May 2012; 6(4):671-673.
4. S V Lavanya, D Jogalakshmi. Asymptomatic bacteriuria in antenatal women. Indian J Med Microbiol. 2002; 20(2):105-106.
5. Longo, Fauci, Kasper, Hauser, Jameson, Loscalzo. Harrison's Principles of Internal Medicine.18th edition, volume II, chapter 288, page 2388.
6. R Sujatha, Manju nawani. Prevalence of asymptomatic bacteriuria and its antibacterial susceptibility pattern among pregnant women attending the antenatal clinic at Kanpur, India. J Clin Diagn Res. Apr 2014; 8(4): DC01-

DC03.

7. T. Jeyaseelan Senthinath, P. Chitra Rajalakshmi, R. Keerthana, R.S.Vigneshwari, P.Revathi et al. Prevalence of asymptomatic bacteriuria among antenatal women in rural tertiary care hospital, Tamilnadu, India. Int.J.Curr.Microbiol.App.Sci. 2013; 2(1):80-85.

8. Annie Rajaratnam, Neha Maria Baby, Thomas. S.Kuruvilla, Santhosh Machado. Diagnosis of asymptomatic bacteriuria and associated risk factors among pregnant women in Mangalore, Karnataka, India. J Clin Diagn Res. Sep 2014; 8(9):OC23-OC25.

9. Santogita Jain, Jaydev Pandya, Sanjay Mehta, Anil Jain, Kunjan Kikani et al. Evaluation of asymptomatic bacteriuria in antenatal women at Cu Shah Medical College and Hospital, Surendranagar, Gujarat. International Journal of Medical Science and Public Health. 2013; 2(4):1021-1023.

10. Chandel Lata R., Kanga Anil, Thakur Kamlesh, Mokta Kiran K, Sood Anuradha et al. Prevalence of pregnancy associated asymptomatic bacteriuria: Astudy done in a Tertiary care hospital .The journal of Obstetrics and Gynecology of India. Sep-Oct 2012; 62(5):511-514.

11. Sudha Birader Kerure, Rajeswari Surpur, Sheela S Sagarad, Sneha Hegadi. Asymptomatic bacteriuria among pregnant women. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. Jun 2013;2(2):213-216.

12. Rajshekhar D Kerure, Umashanker. Prevalence of Asymptomatic bacteriuria among pregnant women in a tertiary care hospital. International journal of scientific and research publications. Nov 2013;3(11):1-4.

13. Raul Raz. Asymptomatic bacteriuria. Clinical significance and management. International Journal of Antimicrobial agents. 22(2003) S45-S47.

14. J C Graham, A Galloway. The laboratory diagnosis of urinary tract infection. J Clin Pathol. 2001;54:911-919.

15. Babita, Sanjeev Suman, Shankar Prakash. Hospital Based Study For Asymptomatic Bacteriuria In Pregnant Women. Journal of evolution of Medical and dental sciences. Nov 2013;2(44):8574-8577.

16. AV Shetty, SH Kumar, M Shekar, AK Shetty, I Karunasagar. Prevalence of adhesive genes among uropathogenic Escherichia coli strains isolated from patients with urinary tract infection in Mangalore. Indian J Med Microbiol.2014;32 (2):175-178.

17. J Jayalakshmi, V.S.Jayaram. Evaluation of various screening tests to detect asymptomatic bacteriuria in pregnant women. Indian Journal of Pathology and Microbiology. Jul – Sep 2008;51(3):379-381.

18. Mandell L. Gerald, Benett E.John, Dolin.R, Mandell Douglas, Benett. Principle and practices of Infectious Diseases. seventh edition. Chapter -Urinary tract infections page 957-983.

19. Collee JG, Miles RS, Watt B (1996). Urinary tract infections. Mackie and McCartney practical medical microbiology. 14th edition. Churchill Livingstone, New York, page 84-90.

20. Geoffrey Chamberlain, Philip Steer. Urinary tract in pregnancy. Turnbulls Obstetrics, Chapter 23, 3rd edition. Page 383-387.

21. Sabahat Saeed and Perween Tariq. Symptomatic and Asymptomatic urinary tract infections during pregnancy. International journal of Microbiological research. 2011;2(2):101-104.

22. F.Gary Cunningham, Kenneth J.Leveno, Steven L. Bloom, John C. Hauth, Larry.C.Gilstrap III, Katharine D.Wenstrom. Renal and Urinary tract disorders. William's Obstetrics, Chapter 48, 22nd edition.page 1093-1099.

23. Patricia M.Tille, Infections of the Urinary tract. Bailey & Scott's Diagnostic microbiology, Chapter 73, 13th edition. page 920.

24. Lindsay E. Nicolle. Asymptomatic bacteriuria : review and discussion of the IDSA guidelines. International Journal of Antimicrobial Agents. 28S (2006) S42-S48.

25. Smaill FM, Vazquez JC. Antibiotics for asymptomatic bacteriuria in pregnancy (Review). The Cochrane library. 2007, Issue 2.

26. J Schnarr, F. Smaill. Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy. European Journal of Clinical Investigation. October 2008; 38 (2): 50-57.

27. Lindsay E. Nicolle, Suzanne Bradley, Richard Colgan, James C. Rice, Anthony Schaeffer et al. Infectious Diseases of America Guidelines for the Diagnosis and treatment of asymptomatic bacteriuria in adults. Clinical Infectious Diseases. March 2005;40:643-654.

28. Anandhi Kasinathan, Prasad Thirumal. Prevalence of Asymptomatic bacteriuria in antenatal women attending a tertiary care hospital. Int J Reprod Contracept Obstet Gynecol.2014 Jun;3(2):437-441.

29. Fiona Smaill. Asymptomatic bacteriuria in pregnancy. Best practice and research clinical obstetrics and gynecology. 2007;21(3):439-450.

30. Elmer W. Koneman, Allen S D, Janda, W M, Schreckenberger PC, Medical bacteriology: Taxonomy, Morphology, Physiology, and virulence. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, Chapter 5, 6th edition, page 200-205.

31. S Ethel, GK Bhat, BM Hedge. Bacterial adherence and humoral immune response in women with symptomatic and asymptomatic urinary tract infection. Indian J Med Micribiol. 2006;24(1):30-33.

32. S.Peter Borriello, Patrick R.Murray, Guido Funke, Hodder Arnold. Bacterial infections of the Urinary tract. Topley and Wilson's Microbiology and Microbial infections. Bacteriology, Chapter 26, 10th edition, page 672-680.

33. R Wasfi, OA Abd El-Rahman, LE Mansour, AS Hanora, AM Hashem et al. Antimicrobial activities against biofilm formed by Proteus mirabilis isolates from wound and urinary tract infection. Indian J Med Micribiol. 2012;30(1):76-80.

34. Rebecca Naveen, Elizabeth Mathai. Some virulence characteristics of Uropathogenic Escherichia coli in different patient groups. Indian J Med Res. 122, August 2005, pp 143-147.

35. Carlos J Sanchez, Katrin Mende, Miriam L Beckius, Kevin S Akers, Desiree R Romano. Biofilm formation by clinical isolates and the implications in chronic infections. BMC Infectious Diseases. 2013; 13(47):1-12.

36. Akira Takahashi, Sojun Kanamaru, Hisao Karuzono, Yasuharu Kunishima, Taiji Tsukamoto et al. Escherichia coli isolates associated with uncomplicated and complicated cystitis and asymptomatic bacteriuria possess similar phylogenies, virulence genes, and O-serogroup profiles. Journal of clinical microbiology. Dec 2006;44(12):4589-4592.

37. Kheya Mukherjee, Saroj Golia, Vasundha CL, Babita, Debojyoti Bhattacharjee et al. A study on asymptomatic bacteriuria in pregnancy: prevalence, etiology and comparision of screening methods. Int J Res Med Sci. 2014 Aug;2(3):1085-1091.

38. Ananthnarayana R, Paniker J. Urinary Tract Infections. Ananthanarayanan and Paniker's text book of Microbiology, Chapter 76, 9th edition, page 670-672.

39. Aziz marjan khattak, Habbibullah khan, Wasim Akhtar, Ihsanullah Mahsud, Bushra. Accuracy of Griess nitrite test to predict Asymptomatic bacteriuria during pregnancy. Gomal Journal of Medical Sciences. Jan-June 2004;2(1):20-23.

40. Sushama S. Thakre, Supriya S. Dhakne, Subash B. Thakre, Amol D.Thakre, Suresh M. Ughade et al. Can the Griess Nitrite test and a Urinary pus cell count of ≥5 cells per micro litre of urine in pregnant women be used for the screening or the early detection of urinary tract infections in rural India?. J Clin Diagn Res. 2012;6(9):1518-1522.

41. Vaishali Jain, Vinita Das, Anjoo Agarwal, Amita pandey, Asymptomatic bacteriuria and obstetric outcome following treatment in early versus late pregnanacy in north Indian women. Indian J Med Res. Apr 2013;137(4):753-758.

42. Maria Jose Munoz-Davila. Role of old antibiotics in the era of antibiotic resistance. Highlighted Nitrofurantoin for the treatment of lower urinary tract infections. Antibiotics 2014;3:39-48.

43. Ekadashi R Sabharwal. Antibiotic susceptibility patterns of uropathogens in Obstetric patients. N Am J Med Sci. Jul 2012;4(7):316-319.

44. Geo. F. Brooks, Karen C. Carroll, Janet S. Butel, Stephen A. Morse, Timothy A.Mietzner. Antimicrobial chemotherapy. Jawetz, Melnick & Adelberg's Medical Microbiology 26/E, Chapter 28, pg 371-405.

45. Shannon M. Moroney, Loree C Heller, Jesse Arbuckle, Monica Talavera, Ray H Widen. Staphylococcus aureus cassette chromosome mec and panton valentine leukocidin characterisation of Methicillin Resistant Staphylococcus aureus clones. J. Clinical Microbiology. May 2007 ; 45 (3): 1019-21.

46. M Gupta, NP Singh, A Kumar, IR Kaur. Cefoxitin disk diffusion test- Better predictor of methicillin resistance in Staphylococcus aureus. Indian J Med Microbiol. 2009 27(4): 379-380.

47. Goodmann and Gillman's. The pharmacological basis of therapeutics. 10th edition, pg 118.

48. Poovendran Ponnusamy, Ramanathan Nagappan. Extended spectrum beta-lactamase, Biofilm producing Uropathogenic Pathogens and their Antibiotic Susceptibility patterns from Urinary tract infection- An overview. International Journal of Microbiological Research. 2013; 4(2):101-118.

49. Bush K, G. A. Jacoby, A.A. Medeiros. A functional classification scheme for β-lactamases and its correlation with molecular structures. Antimicrob Agents Chemother.1995;39(6):1211-1233.

50. Sawai T, S.Mitsuhashi and S.Yamagishi.1968. Drug resistance of enteric bacteria.XIV. Comparison of β-lactamases in gram-negative rod bacteria resistant to α-aminobenzyl penicillin. Jpn.J.Microbiol.12:423-434.

51. Taneja N, Sharma M. ESBLs detection methods in clinical microbiology: Why and How. Indian J Med Res. April 2008;127:297-300.

52. Rudresh SM and Nagarathnamma T. Extended spectrum beta-lactamase producing Enterobacteriaceae and antibiotic co-resistance. Indian J Med Res. Jan 2011;133:116-118.

53. Ankur Goyal, Amit Prasad, Ujjala Ghoshal, K.N. Prasad. Comparison of disk diffusion, disk potentition and double disk synergy methods for detection of extended spectrum beta lactamases in Enterobacteriaceae. Indian J Med Res. August 2008;128:209-211.

54. Clinical and Laboratory Standard Institute. 2014. Performance standards for antimicrobial susceptibility testing; 24th informational supplement. CLSI document M100-S24. Clinical and Laboratory Standard Institute, Wayne, PA.

55. Gaurav Dalela. Prevalence of Extended spectrum beta lactamases Producers among Gram Negative Bacilli from Various Clinical Isolates in a tertiary care hospital at Jhalawar, Rajasthan, India. J Clin Diagn Res. 2012; 6(2):182-187.

56. Paterson DL, Bonomo RA. Extended spectrum beta lactamases: a clinical update. Clin Microbiol.Rev. August 2005;18(4):657-68.

57. I.T.M.A.Overdevest, I.Willemsen, S.Elberts, C.Verhulst, J.A.J.W.Kluytmans Laboratory Detection of Extended Spectrum Beta Lactamase producing Enterobactericeae: Evaluation of two screening agar plates and two confirmation techniques. J Clin Microbiol. 2011 February;49(2):519-522.

58. Cheesbrough, M. Examination of urine. District Laboratory Practice in Tropical countries. Part 2, Chapter 7.12, page 105-115.

59. Douglas G Tincello. Evaluation of reagent strips in detecting asymptomatic bacteriuria in early pregnancy. British Medical Journal. 1998; 316:435.

60. A Titoria, A M Rathore, A Gupta, S K Prakash, D Rawat et al. Asymptomatic bacteriuria in women attending an antenatal clinic at a tertiary care centre. S Afr J OG. 2014;20(1):4-7.

61. Gayathree L, Shetty S, Deshpande S R, Venkatesha D T. Screening for Asymptomatic bacteriuria in pregnancy: An evaluation of various screening tests at the Hassan District Hospital, India. J Clin Diagn Res. 2010 August;(4):2702-2706.

62. Lindsay E.Nicolle. Asymptomatic bacteriuria When to screen and when to treat. Infect Dis Clin N Am. 17 (2003) 367-394.

63. S Sharma, GK Bhat, S Shenoy. Virulence factors and drug resistance in Escherichia coli isolated from extra intestinal infections. Indian J Med Microbiol. 2007; 25(4):369-73.

64. Sunanda N Shrikhande, Chhaya A Chande, A A Pathak. Virulence factors in Uropathogenic E.coli. Indian J. Pathol. Microbiol.1999; 42(3): 321-325.

65. Saroj Golia, Vivek Hittinahalli, Sujatha K Karjigi, Mallika Reddy. Correlation between biofilm formation of uropathogenic Escherichia coli and its antibiotic resistance pattern. Journal of evolution of Medical and Dental Sciences. July-Sept 2012;1(3):166 -175.

66. Francisco Toval, Christian Daniel Kohler, Ulrich Vogel, Florian Wagenlehner, Alexander Mellmann et al. Characterization of Escherichia coli isolates from Hospital inpatients or outpatients with urinary tract infection. Journal of clinical microbiology. Feb 2014;52(2):407-418.

67. Jean- Philippe Lavigne, Adeline Boutet Dubois, Dorsaf Laouini, Christophe Combescure, Nicole Bouziges et al. Virulence potential of Escherichia coli strains causing Asymptomatic bacteriuria during pregnancy. Journal of clinical microbiology. Nov 2011;49(11): 3950-3953.

68. M Mishra, YS Thakar, AA Pathak. Haemagglutination, Hemolysin production and serum resistance of Proteus and related species isolated from clinical sources. Indian J Med Microbiol. 2001;19(2): 91-96.

69. Lars E.P.Dietrich, Alexa Price- Whelan, Ashley Petersen, Marvin Whiteley, Dianne K.Newman. The Phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of Pseudomonas aeruginosa. Molecular Microbiology. 2006; 61(5):1308-1321.

70. P Gupta, RK Gupta, K Harjai. Multiple virulence factors regulated by quorum sensing may help in establishment and colonisation of urinary tract by Pseudomonas aeruginosa during experimental urinary tract infection. Indian J Med Microbiol. 2013;31(1): 29-33.

71. Praharaj Ira, Sistla Sujatha, Parija Subhash Chandra. Virulence factors in clinical and commensal isolates of Enterococcus species. Indian J Pathol Microbiol. 2013; 56(1): 24-30.

72. Niranjan V, Malini A. Antimicrobial resistant pattern in Escherichia coli causing urinary tract infection among inpatients. Indian J Med Res. 139, June 2014; pp 945-948.

73. Bauer AW, Kirby WMM, Sherris JC, Tuck M. Antibiotics susceptibility testing by standardized single disc method. Am J Clin Pathol.1996; 45: 493-6.

74. Anand K B, Aggarwal P, Kumar S, Kapila K. Comparison of Cefoxitin disk diffusion test, Oxacillin screen agar and PCR for Mec A gene for detection of MRSA. Indian J Med Microbiol.2009;27(1):27-29.

75. Umadevi S, Kandhakumari G, Joseph N M, Kumar S, Easow J M et al. Prevalence and antimicrobial susceptibility pattern of ESBL producing Gram negative bacilli. J Clin Diagn Res. Apr 2011;5(2):236-239.

76. S Babypadmini, B Appalaraju. Extended spectrum beta-lactamases in urinary isolates of Escherichia coli and Klebsiella pneumonia- Prevalence and susceptibility pattern in a tertiary care hospital. Indian J Med Microbiol. 2004;22(3): 172-174.

77. B Nandagopal, S Sankar, K Sagadevan, H Arumugam, MV Jesudason et al. Frequency of Extended spectrum beta-lactamase producing urinary isolates of Gram-negative bacilli among patients seen in a multispeciality hospital in Vellore District, India. Indian J Med Microbiol. 2015; 33(2): 282-285.

78. Mandira Mukherjee, Shreya Basu, Sandip kumar mukherjee, Monalisa Majumder. Multidrug- Resistance and extended spectrum beta- lactamase

production in Uropathogenic E. coli which were isolated from hospitalised patients in Kolkata, India. J Clin Diagn Res. March 2013;7(3):449-453.

79. G. Revathi. Detection of Extended spectrum Beta Lactamases using E-Test ESBL Strip. Indian J Med Microbiol.1997;15(2):69-71.

80. Anandkumar Harwalkar, Jagadeesh Sataraddi, Soham Gupta, Raksha Yoganand, Achut Rao et al. The detection of ESBL producing Escherichia coli in patients with symptomatic urinary tract infections using different diffusion methods in a rural setting. Journal of Infection and Public health. 2013; 6:108-114.

81. Dallene C, Da Costa A, Decre D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. J Antimicrob Chemother. Mar 2010;65:490-495.

82. R J Girishbabu, R Srikrishna, S T Ramesh. Asymptomatic bacteriuria in pregnancy. Int J Biol Med Res. 2011;2(3):740-742.

83. J Agarwal, S Srivastava, M Singh. Pathogenomics of uropathogenic Escherichia coli. Indian J Med Microbiol. 2012;30(2)141-149.

84. Rohini P, Ambica R, Nagarathnamma T. The study of virulence factor production in Escherichia coli causing urinary tract infections. International Journal of Current Research. Sep 2013;5(9):2613-2616.

85. Imade PE, Izekor PE, Eghafona NO, Enabulele OI, Ophori E. Asymptomatic bacteriuria among pregnant women. North Am J Med Sci. June 2010;2:263-66.

86. Afreenish Hassan, Javaid Usman, Fatima Kaleem, Maria Omair, Ali Khalid et al. Evaluation of Different Detection methods of Biofilm formation in the clinical isolates. Braz J Infect Disease. 2011:15(4):305-311.

87. Ajaye AB, Nwabuisi C, Aboyeji AP, Ajayi NS, Fowotade A et al. Asymptomatic bacteriuria in antenatal patients in Ilorin, Nigeria. Oman Med J. 2012;27(1):31-35.

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S.No	OP. No	Age	Gravida	Trimester	GA	PUS CELLS	Direct Gram stain	Nitrite	Leucocyte Esterase	ORGANISM	hemolysin	Cell surface hydrophobicity	Serum resistance	Gelatinase	MRHA	Biofilm	Capsule	Pyocyanin	Slide coagulase	Tube coagulase	DNase	Urease	Phosphatase	AMX	AMC	CN	CTX	CAZ	AK	NIT	NX	GEN	OF	COT	PIT	IMP	VA	CX	ESBL
1	5265	26	G2P1L1	II	clear	+	-	-	-	NG																														
2	1027	28	Primi	III	clear	-	-	+	-	NG																														
3	5252	23	G2P1L1	III	clear	-	-	-	+	NG																														
4	1009	34	Primi	II	clear	-	-	-	-	NG																														
5	1024	24	Primi	III	turbid	+	-	+	+	Escherichia coli		+	+		+									R	S	R	R	R	S	S	R	S	R	S	S	S			ESBL
6	1018	24	Primi	I	clear	-	-	-	-	NG																														
7	852	21	Primi	III	clear	-	-	-	+	NG																														
8	90	25	Primi	II	clear	-	-	-	+	NG																														
9	1097	24	Primi	II	clear	+	+	+	-	Staphylococcus aureus	+								+	+	+	+	+	R	R	R	S		S	S	S	R	S	S			S	S	
10	864	22	Primi	II	clear	-	-	-	-	NG																														
11	992	27	G3P2L1	III	clear	-	-	-	+	NG																														
12	1144	21	Primi	III	clear	+	-	-	-	NG																														
13	1021	19	Primi	III	turbid	-	-	-	-	NG																														
14	1114	34	G2P1L1	II	clear	-	-	-	-	NG																														
15	1145	30	G2P1L1	III	turbid	+	+	+	+	Klebsiella pneumoniae							+							S	R	S	S	S	S	S	R	S	S	S	S	S			
16	4842	25	G2P1L1	III	clear	-	-	-	+	NG																														
17	1112	28	G3P1L1A1	III	clear	-	-	-	-	NG																														
18	1134	29	G3P2L2	III	clear	-	-	-	+	NG																														
19	1217	30	G2P1L1	III	clear	-	-	-	-	NG																														
20	1215	23	Primi	III	turbid	-	-	-	-	NG																														
21	332	22	G2P1L1	II	clear	-	+	-	-	NG																														
22	868	22	G2P1L1	III	clear	-	-	-	+	NG																														
23	1206	24	G2P1L1	III	clear	-	-	-	-	NG																														
24	1239	18	Primi	II	clear	-	-	-	-	NG																														
25	5574	30	G2P1L1	II	turbid	-	-	-	-	NG																														
26	3308	22	Primi	II	clear	-	-	-	-	NG																														
27	1227	25	Primi	II	clear	+	-	-	+	NG																														
28	1229	29	G3P2L2	II	clear	-	-	-	-	NG																														

29	883	25	G2P1L1	II	clear	-	-	-	-	NG																																
30	1239	28	G2P1L1	III	clear	-	-	-	-	NG																																
31	1821	26	Primi	II	turbid	+	+	+	-	Klebsiella pneumoniae						+	+							R	S	R	S	S	S	S	S	S	S	S	S	S			S	S	
32	1165	23	G2P1L1	III	clear	-	-	-	-	NG																																
33	984	20	Primi	II	clear	-	-	-	-	NG																																
34	539	25	G2P1L1	III	clear	-	-	-	-	NG																																
35	1340	21	Primi	II	clear	-	-	-	+	NG																																
36	143	24	G2P1L1	III	clear	-	-	-	-	NG																																
37	1338	25	G3P1L1A1	I	clear	-	-	-	+	NG																																
38	6058	25	Primi	III	clear	-	-	-	-	NG																																
39	1263	21	Primi	II	clear	-	-	-	-	NG																																
40	1372	28	G2P1L1	III	clear	+	+	-	+	Pseudomonas aeruginosa	+							+							S			S	R	S	R	R	S	S	S	S			S	S	
41	786	28	G2P1L1	III	clear	-	-	-	-	NG																																
42	1380	23	G4P3L3	III	clear	+	-	-	-	NG																																
43	671	22	G2p1L0	II	clear	-	-	-	-	NG																																
44	1381	22	G2P1L1	II	clear	-	-	-	-	NG																																
45	1379	21	G2PILI	III	turbid	-	-	-	-	NG																																
46	671	29	Primi	III	clear	-	-	-	-	NG																																
47	1246	25	G3PoLoA2	II	clear	-	-	-	-	NG																																
48	1324	26	Primi	III	turbid	-	-	-	-	NG																																
49	1370	22	Primi	I	clear	-	-	-	-	NG																																
50	292	38	G4P2L2A1	II	clear	-	-	-	-	NG																																
51	1275	28	G2P1L1	II	clear	-	-	-	+	NG																																
52	2450	24	Primi	III	clear	-	-	-	-	NG																																
53	332	22	G2P1l1	III	turbid	+	+	-	+	Staphylococcus aureus	+								+	+	+	+	+	R	S	S	S		S	S	R	S	S	S			S	S	
54	1432	29	Primi	III	clear	-	-	-	-	NG																																
55	1406	20	Primi	III	clear	+	-	-	-	NG																																
56	2367	21	Primi	III	clear	+	+	+	-	Escherichia coli		+	+	+										S	S	S	S	S	S	S	S	R	S	S	S	S				
57	1965	32	G4P3A2L1	III	clear	-	-	-	-	NG																																
58	2134	24	G2P1L1	III	clear	-	-	-	+	NG																																
59	2458	25	Primi	III	clear	-	-	-	-	NG																																

60	2459	27	Primi	I	clear	-	-	-	-	NG																																
61	2456	22	Primi	III	clear	-	-	-	+	NG																																
62	3488	23	Primi	III	clear	-	-	-	-	NG																																
63	2980	23	Primi	III	clear	-	-	-	-	NG																																
64	241	23	G2P1L1	III	clear	-	-	-	-	NG																																
65	2338	22	Primi	III	clear	-	-	-	-	NG																																
66	842	31	G2P1L1	III	clear	-	-	-	-	NG																																
67	2540	25	G3P2L2	III	clear	+	+	+	-	Escherichia coli	+	+											S	S	S	S	S	R	S	S	R	S	R	S	S						
68	425	24	G3P2L1	III	clear	-	-	-	-	NG																																
69	1046	30	G2P1L1	III	clear	-	-	-	-	NG																																
70	2559	23	Primi	III	clear	-	+	-	-	NG																																
71	2560	22	Primi	III	clear	-	-	-	-	NG																																
72	274	25	G2P1L1	II	clear	-	-	-	+	NG																																
73	1464	25	Primi	II	clear	+	-	-	-	NG																																
74	1353	26	Primi	I	turbid	-	-	-	-	NG																																
75	4589	25	Primi	II	clear	-	-	-	-	NG																																
76	1362	32	G2P1L1	III	clear	-	-	-	-	NG																																
77	1786	21	G3P2L2	III	clear	-	-	-	-	NG																																
78	1142	34	Primi	III	clear	-	-	-	-	NG																																
79	421	21	Primi	I	clear	-	-	-	-	NG																																
80	2312	31	Primi	II	clear	-	-	-	+	NG																																
81	1713	20	G2P1L1	II	clear	+	-	-	-	NG																																
82	2523	24	G2P0L0A1	II	clear	-	-	-	-	NG																																
83	1217	27	G3P2L2	II	clear	-	-	-	-	NG																																
84	2712	29	Primi	II	clear	-	-	-	-	NG																																
85	1116	18	G2P1L1	III	clear	-	-	-	-	NG																																
86	423	28	G3P0L0A2	I	turbid	+	-	+	+	Escherichia coli													R	S	R	S	S	R	R	R	R	S	S	S	S						
87	2132	18	Primi	II	clear	-	-	-	-	NG																																
88	1129	27	Primi	I	clear	-	-	-	-	NG																																
89	829	24	G2P1L1	II	clear	-	-	-	-	NG																																
90	525	21	G2P0L0A1	III	clear	-	-	-	-	NG																																

91	323	26	PRIMI	III	clear	-	+	-	-	NG																																	
92	1232	25	G2P1L1	II	clear	-	-	-	-	NG																																	
93	1118	23	Primi	II	clear	-	-	+	-	NG																																	
94	3142	20	G2P1L1	II	clear	+	-	-	-	NG																																	
95	2231	19	Primi	II	clear	-	-	-	-	NG																																	
96	1436	26	G3P2L2	III	clear	-	+	-	-	NG																																	
97	1217	26	Primi	I	clear	-	-	-	-	NG																																	
98	2541	22	G2P1L1	II	clear	-	-	-	-	NG																																	
99	2330	20	Primi	II	clear	-	-	-	-	NG																																	
100	2117	19	Primi	II	turbid	-	-	-	-	NG																																	
101	3164	27	Primi	III	turbid	-	-	-	-	NG																																	
102	3163	20	Primi	III	clear	-	-	-	-	NG																																	
103	3144	23	Primi	II	clear	-	-	-	-	NG																																	
104	3161	28	G2P1L1	III	clear	-	-	-	-	NG																																	
105	3165	20	G2P1L1	II	clear	-	-	-	-	NG																																	
106	843	23	Primi	II	turbid	+	-	+	-	Escherichia coli			+	+		+								S	S	S	S	S	S	S	S	S	S	S	S	S					
107	3166	29	G2P1L1	III	clear	-	-	-	-	NG																																	
108	933	24	G2P1L0	II	clear	-	-	-	-	NG																																	
109	2371	30	Primi	III	clear	-	-	-	-	NG																																	
110	967	19	Primi	II	clear	+	-	-	-	NG																																	
111	2127	25	G2P1L1	I	clear	-	-	-	-	NG																																	
112	3153	25	Primi	III	clear	-	-	-	-	NG																																	
113	2424	23	Primi	III	clear	-	-	-	-	NG																																	
114	3177	21	Primi	II	clear	-	-	-	-	NG																																	
115	2315	25	G2P1L1	I	clear	-	-	-	-	NG																																	
116	3176	21	Primi	III	clear	-	-	-	-	NG																																	
117	1559	22	G2P1L1	III	clear	-	-	-	-	NG																																	
118	660	24	G3P2L1A0	II	clear	-	-	-	-	NG																																	
119	378	23	G3P2L2	III	clear	-	-	-	-	NG																																	
120	2247	22	Primi	III	clear	-	-	-	-	NG																																	
121	1780	22	Primi	III	clear	-	-	-	-	NG																																	

122	2219	30	G2P1L1	II	clear	-	+	-	-	NG																																
123	2418	27	G2P1L1	II	clear	+	+	+	+	Pseudomonas aeruginosa						+		+							S			S	S	S	R	S	S	R	S	S				
124	2457	23	Primi	III	clear	-	-	-	-	NG																																
125	2569	23	G2P1L1	III	clear	-	-	-	-	NG																																
126	3795	29	G2P1L1	III	clear	+	-	+	-	Klebsiella pneumoniae							+							S	R	S	S	S	S	R	R	R	R	R	S	S				
127	3338	26	G2P1L1	III	clear	-	-	-	-	NG																																
128	4310	27	G2P1L1	III	clear	-	-	-	+	NG																																
129	1420	25	G2P1L1	III	clear	-	-	-	-	NG																																
130	1299	24	G3P2L1	III	clear	-	-	-	-	NG																																
131	2243	23	G2P1L1	III	turbid	+	+	+	-	Klebsiella pneumoniae							+							S	S	R	S	S	S	S	S	R	S	S	S	S				
132	2677	22	G2P1L1	III	turbid	+	+	-	-	Staphylococcus aureus	+								+	+	+	+	+	R	S	S	S		R	S	S	R	S	R			S	S	
133	3320	26	G2P1L1	III	clear	-	-	-	-	NG																																
134	2499	29	G3P2L2	III	clear	-	-	-	-	NG																																
135	2567	22	Primi	II	clear	+	-	-	-	NG																																
136	3108	27	Primi	III	turbid	-	-	-	-	NG																																
137	2571	37	G3P1L1A1	III	clear	-	-	-	+	NG																																
138	1431	21	G2P1L1	II	clear	-	-	-	-	NG																																
139	4571	24	G2P1L1	III	clear	-	-	-	-	NG																																
140	3210	22	Primi	III	clear	-	-	-	-	NG																																
141	3342	23	G2P1L1	III	clear	-	-	-	-	NG																																
142	2189	22	Primi	III	clear	-	-	-	-	NG																																
143	3409	26	G2P1L1	II	clear	-	-	-	-	NG																																
144	4310	24	G2P1L1	III	clear	-	-	-	-	NG																																
145	3570	25	G2P1L1	III	clear	-	-	-	-	NG																																
146	4313	26	Primi	II	clear	-	-	-	-	NG																																
147	2139	26	Primi	II	clear	-	-	-	-	NG																																
148	1268	30	Primi	III	clear	-	-	-	-	NG																																
149	3299	32	G3P2L2	II	clear	-	-	-	-	NG																																
150	1350	19	Primi	I	clear	-	-	-	-	NG																																
151	2370	27	Primi	III	clear	-	-	+	-	NG																																
152	3236	21	Primi	III	clear	-	-	-	-	NG																																

153	3157	27	G2P1L1	III	clear	-	-	-	-	NG																																		
154	2471	24	Primi	III	turbid	+	+	+	+	Staphylococcus aureus	+								+	+	+	+	+	R	S	R	R		S	R	R	S	S	R			S	S					
155	1623	22	Primi	III	clear	-	-	-	-	NG																																		
156	2231	25	G2P1L1	III	clear	-	-	-	-	NG																																		
157	326	23	Primi	III	turbid	+	-	+	-	Escherichia coli	+			+		+								R	S	S	R	R	S	S	S	S	S	S	S	S			ESBL			
158	2162	20	G2P1L1	I	clear	-	-	-	-	NG																																		
159	2179	27	Primi	II	clear	-	-	-	-	NG																																		
160	4691	31	G2P1L1	I	clear	-	-	-	-	NG																																		
161	3239	20	Primi	II	clear	+	-	-	-	NG																																		
162	1542	19	Primi	II	clear	-	-	-	-	NG																																		
163	3241	25	Primi	I	clear	-	-	-	-	NG																																		
164	906	28	Primi	II	clear	-	-	-	-	NG																																		
165	3235	23	Primi	II	clear	-	-	-	-	NG																																		
166	3243	25	G2P1L1	II	clear	-	-	-	-	NG																																		
167	3242	19	Primi	II	clear	-	-	-	-	NG																																		
168	3225	25	Primi	I	clear	+	+	-	-	Staphylococcus aureus						+			+	+	+	+	+	R	S	R	S		S	R	R	S	S	S			S	S					
169	2389	22	G2P1L1	I	clear	-	-	-	-	NG																																		
170	1398	19	Primi	I	clear	-	-	-	-	NG																																		
171	2985	22	G2P1L1	I	clear	-	-	-	-	NG																																		
172	4431	20	Primi	I	clear	+	+	+	+	Staphylococcus saprophyticus						+								S	S	S	S		S	S	R	S	S	S			S	S					
173	3233	24	G3P1L1A1	II	clear	-	-	-	-	NG																																		
174	3512	27	G2P1L1	III	clear	-	-	-	-	NG																																		
175	2500	19	Primi	I	clear	-	-	-	-	NG																																		
176	2167	25	Primi	III	clear	-	-	-	-	NG																																		
177	7175	24	G3P2L2	III	clear	-	-	-	-	NG																																		
178	3152	21	Primi	III	clear	-	-	-	-	NG																																		
179	3290	26	G2P1L1	III	clear	-	-	-	-	NG																																		
180	2078	24	G2P1L1	I	clear	-	-	-	-	NG																																		
181	5680	23	G2P1L1	III	turbid	+	+	+	-	Escherichia coli					+									S	S	S	S	S	S	S	R	S	S	R	S	S							
182	2109	20	Primi	II	clear	-	-	-	-	NG																																		
183	3412	20	Primi	III	clear	-	-	-	-	NG																																		

184	5610	28	G2P1l1	III	clear	-	-	-	-	NG																																		
185	2168	27	Primi	III	clear	-	-	-	-	NG																																			
186	1157	21	Primi	III	clear	-	-	-	-	NG																																			
187	2095	29	G2P1l1	III	clear	+	+	+	+	Escherichia coli	+	+												S	S	R	S	S	S	S	S	R	S	S	S	S						
188	2150	24	G2P1l1	III	clear	-	-	-	-	NG																																		
189	1879	24	G2P1l1	III	clear	-	-	-	-	NG																																		
190	1753	21	G2P1l1	III	clear	-	-	-	+	NG																																		
191	1849	20	G2P1l1	III	clear	-	-	-	-	NG																																		
192	1597	25	Primi	III	clear	+	+	-	+	Klebsiella pneumoniae						+	+							S	S	R	S	S	S	S	R	S	S	S	S	S						
193	2156	28	G2P1l1	III	clear	-	-	-	-	NG																																		
194	2164	19	Primi	I	clear	-	-	-	-	NG																																		
195	1054	21	G2P1l1	II	clear	-	-	-	-	NG																																		
196	3210	30	G2P1L1	II	clear	-	-	-	-	NG																																		
197	2241	22	G3P2L2	II	clear	-	-	-	-	NG																																		
198	2154	28	Primi	III	clear	-	-	-	-	NG																																		
199	1742	28	Primi	II	clear	-	-	-	-	NG																																		
200	1748	37	G3P2L2	III	clear	-	-	-	-	NG																																		
201	4532	23	G2P1L1	II	clear	+	-	-	-	NG																																		
202	2139	22	G2P1L1	II	clear	-	-	-	-	NG																																		
203	4509	23	Primi	III	clear	-	-	-	-	NG																																		
204	3217	19	Primi	III	clear	-	-	-	-	NG																																		
205	4446	26	G2P1L1	II	clear	-	-	-	-	NG																																		
206	3489	29	G2P1L1	I	clear	-	-	-	-	NG																																		
207	3399	20	Primi	III	clear	-	-	-	-	NG																																		
208	4410	28	G2P1L1	II	clear	-	-	-	-	NG																																		
209	5217	29	G6A5	III	clear	-	-	-	-	NG																																		
210	2970	30	Primi	II	turbid	+	-	-	-	Staphylococcus aureus						+			+	+	+	+	+	R	S	R	S		R	S	R	R	R	R			S	S		
211	4088	23	Primi	II	clear	-	-	-	-	NG																																		
212	2468	23	G2P1L1	I	clear	-	-	-	-	NG																																		
213	3371	21	G2P1L1	III	clear	-	-	-	-	NG																																		
214	1882	25	Primi	II	clear	-	-	-	-	NG																																		

215	3502	28	G4A3	II	clear	+	+	+	-	Staphylococcus aureus	+								+	+	+	+	+	R	S	S	R		S	S	S	S	S	S			S	S	
216	3507	22	G2P1L1	III	clear	-	-	-	-	NG																																
217	3511	25	G3P2L2	III	clear	-	-	-	-	NG																																
218	3505	28	G2P1L1	II	clear	-	-	-	-	NG																																
219	3509	25	G2P1L1	III	clear	-	-	-	-	NG																																
220	3510	25	G2P1L1	III	clear	-	-	-	-	NG																																
221	2903	24	Primi	III	clear	-	-	-	-	NG																																
222	3508	23	G3P1L1A1	II	clear	-	-	-	-	NG																																
223	3495	20	Primi	III	clear	-	-	-	-	NG																																
224	3068	27	G3P1L1A1	I	clear	-	-	-	-	NG																																
225	3294	26	G3P2L2	II	clear	+	+	+	-	Klebsiella pneumoniae							+							R	R	R	S	S	R	R	R	S	S	R	S	S				
226	3490	33	G4P1L1A2	II	clear	-	-	-	-	NG																																
227	2997	28	Primi	II	clear	-	-	-	-	NG																																
228	3496	21	G2P1L1	II	clear	-	-	-	-	NG																																
229	3512	24	G2P1L1	II	clear	-	-	-	-	NG																																
230	2813	29	G2P1L1	II	clear	-	-	-	-	NG																																
231	3308	23	G3P1L1A1	II	turbid	+	+	+	+	Klebsiella pneumoniae							+							S	S	R	R	R	R	S	S	R	S	R	S	S				ESBL
232	1551	27	G3P2L2	II	clear	-	-	-	-	NG																																
233	1555	20	Primi	III	turbid	+	-	+	+	Escherichia coli	+					+								R	S	S	S	S	S	S	S	S	S	S	S	S				
234	2341	24	G2P1L1	II	clear	+	-	-	-	NG																																
235	2690	21	Primi	III	clear	-	-	-	-	NG																																
236	1665	23	G2P1l1	II	clear	-	-	-	-	NG																																
237	3287	25	G3P2L2	III	clear	-	-	-	-	NG																																
238	3307	23	G2P1l1	III	turbid	+	-	-	+	Staphylococcus aureus	+					+			+	+	+	+	+	R	S	R	S		R	S	S	R	S	R			S	S	
239	4411	30	G2P1L1	III	clear	-	-	-	-	NG																																
240	4321	28	Primi	II	clear	-	-	-	-	NG																																
241	3492	20	Primi	II	clear	-	-	-	-	NG																																
242	1195	24	Primi	III	clear	-	-	-	-	NG																																
243	1667	21	Primi	II	turbid	-	-	-	-	NG																																
244	2351	25	G2P1L1	II	clear	-	-	-	-	NG																																
245	2247	22	Primi	III	clear	-	-	-	-	NG																																

246	3846	21	Primi	III	clear	-	-	-	-	NG																																
247	3506	27	G2P1L1	III	clear	-	-	-	-	NG																																
248	3838	24	Primi	I	clear	-	-	-	-	NG																																
249	3840	25	G2P1L1	III	clear	-	-	-	-	NG																																
250	2439	24	Primi	II	turbid	+	+	+	-	Escherichia coli	+		+										S	S	S	S	S	R	S	R	S	S	R	S	S					
251	3849	24	G2P1L1	III	clear	-	-	-	-	NG																																
252	3593	20	Primi	II	clear	+	-	-	-	NG																																
253	3593	23	Primi	III	turbid	-	-	-	-	NG																																
254	2940	28	Primi	II	clear	-	-	-	-	NG																																
255	3821	23	Primi	I	clear	-	-	-	-	NG																																
256	3650	28	G2P1L1	III	clear	-	-	-	-	NG																																
257	618	21	Primi	III	clear	-	-	-	-	NG																																
258	2571	29	Primi	III	clear	-	-	-	-	NG																																
259	3851	22	G2P1L1	II	clear	-	-	-	-	NG																																
260	3601	23	G3P1L1A1	II	clear	+	+	+	+	Staphylococcus aureus	+								+	+	+	+	+	R	S	S	S		S	S	R	S	S	R			S	S	
261	3866	25	G2P1L1	III	clear	-	-	-	-	NG																																
262	3867	23	Primi	II	clear	-	-	-	-	NG																																
263	3200	28	Primi	III	clear	-	-	-	-	NG																																
264	3533	21	G2P1L1	I	clear	-	-	-	-	NG																																
265	3670	27	G3P1L1A1	I	clear	-	-	-	-	NG																																
266	2960	26	G2P1L1	I	clear	-	-	-	-	NG																																
267	3217	24	Primi	II	clear	-	-	-	-	NG																																
268	4559	24	Primi	I	clear	-	-	-	+	NG																																
269	3859	26	Primi	I	turbid	-	-	-	-	NG																																
270	2299	20	G2P1L1	II	clear	-	-	-	-	NG																																
271	2588	23	Primi	III	clear	-	+	-	-	NG																																
272	3864	24	Primi	III	turbid	+	+	-	+	Pseudomonas aeruginosa	+					+		+						R			S	R	S	S	R	R	S	S	S					
273	1868	29	G4P1L1A2	II	clear	-	-	-	-	NG																																
274	8863	23	Primi	II	clear	-	-	-	-	NG																																
275	3316	21	Primi	II	clear	-	-	-	-	NG																																
276	4100	22	G2P1L1	III	clear	-	-	-	-	NG																																

277	3460	30	G3P2L2	III	clear	-	-	-	-	NG																																	
278	3004	25	G2P1L1	III	clear	-	-	-	-	NG																																	
279	8374	19	Primi	I	clear	-	-	-	-	NG																																	
280	3311	20	Primi	III	clear	-	-	-	+	NG																																	
281	3812	20	Primi	II	clear	-	-	-	-	NG																																	
282	2368	27	G2P1L1	II	clear	-	-	-	-	NG																																	
283	2778	22	G2P1L1	III	clear	+	-	+	-	Escherichia coli	+				+								S	S	S	S	S	R	S	S	R	S	S	S	S						
284	3372	23	G2P1L1	II	clear	-	-	-	-	NG																																	
285	314	20	Primi	II	clear	-	-	-	-	NG																																	
286	3335	24	Primi	I	clear	-	-	-	-	NG																																	
287	2921	25	G3P1L1A1	II	clear	-	-	-	-	NG																																	
288	1676	26	Primi	II	turbid	+	-	-	-	NG																																	
289	3036	25	G2P1L1	II	clear	-	-	-	-	NG																																	
290	3486	29	G2P1L1	II	clear	-	-	-	-	NG																																	
291	1825	29	G2P1L1	III	clear	-	-	-	+	NG																																	
292	2221	21	Primi	II	clear	-	-	+	-	NG																																	
293	1049	21	Primi	II	clear	-	-	-	-	NG																																	
294	1419	22	Primi	III	clear	-	-	-	-	NG																																	
295	1542	18	Primi	I	clear	-	-	-	-	NG																																	
296	3952	24	G2P1L1	II	clear	+	+	-	+	Enterococcus faecalis				+									S	S				S	S	S	S	S	S			S				
297	3948	24	G2P1L1	I	clear	-	-	-	-	NG																																	
298	3947	21	G2P1L1	III	clear	-	-	-	-	NG																																	
299	2781	26	Primi	III	clear	-	-	-	+	NG																															S	S			
300	3954	24	G2P1L1	III	clear	-	-	-	-	NG																																	
301	3050	26	G2P1L1	III	clear	-	-	-	-	NG																																	
302	2662	22	Primi	III	clear	-	-	-	-	NG																																	
303	707	20	Primi	I	clear	-	-	-	-	NG																																	
304	3955	28	G2P1L1	III	clear	-	-	-	-	NG																																	
305	3950	27	G2P1L1	II	clear	+	-	-	-	NG																																	
306	3958	26	G2P1L1	II	clear	-	-	-	-	NG																																	
307	3956	26	G2P1L1	II	clear	-	-	-	-	NG																																	

308	3813	22	G2P1L1	I	clear	-	-	-	-	NG																													
309	3009	24	G2P1L1	II	clear	-	-	-	-	NG																													
310	3954	20	Primi	I	clear	+	+	+	-	Escherichia coli		+												R	S	S	S	S	S	R	R	S	S	S	S	S			
311	8590	19	Primi	III	clear	-	-	-	-	NG																													
312	2155	28	G2P1L1	II	clear	-	-	-	-	NG																													
313	6018	29	G3P1L1A1	II	clear	-	-	-	-	NG																													
314	8451	31	Primi	II	clear	-	-	-	-	NG																													
315	8481	35	G3P2L2	III	clear	-	-	-	-	NG																													
316	3638	29	G2P1L1	I	clear	-	-	-	-	NG																													
317	3273	19	Primi	I	turbid	-	-	-	-	NG																													
318	7633	30	G2P1L1	III	clear	-	-	-	-	NG																													
319	4165	33	G2P1L1	II	clear	-	-	-	-	NG																													
320	4160	26	G2P1L1	III	clear	-	-	-	-	NG																													
321	2813	22	Primi	III	clear	-	-	-	-	NG																													
322	4166	22	Primi	II	clear	-	-	-	-	NG																													
323	2183	23	G2P1L1	II	clear	+	-	-	-	NG																													
324	2661	20	Primi	III	turbid	+	+	-	+	Staphylococcus aureus	+					+			+	+	+	+	+	R	S	R	R		S	S	S	R	R	S			S	S	
325	3113	29	G2P1L1	II	clear	-	-	-	-	NG																													
326	4073	27	G2P1L1	II	clear	-	-	-	-	NG																													
327	4164	24	Primi	III	clear	-	-	-	-	NG																													
328	4151	27	G2P1L1	II	clear	-	-	-	-	NG																													
329	4113	25	G2P1L1	II	clear	-	-	-	-	NG																													
330	1184	22	G2P1L1	III	clear	-	-	-	-	NG																													
331	2353	23	Primi	II	clear	-	-	-	-	NG																													
332	934	24	G2P1L1	II	turbid	-	-	-	-	NG																													
333	6822	33	G2P1L1	II	clear	+	-	-	-	Staphylococcus aureus							+		+	+	+	+		R	S	S	S		S	R	R	S	S	R			S	S	
334	4168	23	G3p2l2	I	clear	+	+	+	-	Escherichia coli	+					+								S	S	S	S	S	S	S	S	R	S	S	S	S			
335	2864	23	G2P1L1	II	clear	-	-	-	-	NG																													
336	4169	20	G2P1L1	II	clear	-	-	-	-	NG																													
337	8877	20	Primi	I	clear	-	-	-	-	NG																													
338	6984	19	Primi	I	clear	-	-	-	-	NG																													

339	4137	24	G2P1L1	III	clear	-	-	-	-	NG																																	
340	4138	33	G3P2L2	III	clear	-	-	-	-	NG																																	
341	7175	24	Primi	I	clear	-	-	-	-	NG																																	
342	2040	23	Primi	II	clear	-	-	-	-	NG																																	
343	4050	18	Primi	I	clear	+	+	+	-	Klebsiella pneumoniae							+							S	S	S	S	S	S	S	S	S	S	S	S	S					
344	6943	31	G2P1L1	III	clear	+	-	-	-	NG																																	
345	4043	21	Primi	I	clear	-	-	-	-	NG																																	
346	4142	19	Primi	II	clear	-	-	-	-	NG																																	
347	4144	25	G2P0L0A1	II	clear	-	-	-	-	NG																																	
348	2500	19	Primi	II	clear	-	-	-	-	NG																																	
349	1542	18	Primi	II	turbid	+	+	+	+	Escherichia coli	+			+										S	S	R	S	S	R	S	R	R	S	R	S	S					
350	4436	27	G2p1L1	III	clear	-	-	-	-	NG																																	
351	3292	28	G2P0L0A1	I	clear	-	-	-	-	NG																																	
352	3015	23	Primi	II	clear	-	-	-	-	NG																																	
353	4170	20	G2P1L1	II	clear	-	-	-	-	NG																																	
354	4171	23	Primi	II	clear	-	-	-	-	NG																																	
355	3422	22	Primi	II	clear	-	-	-	-	NG																																	
356	2891	24	G2P1L1	II	clear	-	-	-	-	NG																																	
357	4184	25	Primi	II	clear	-	-	-	-	NG																																	
358	2765	21	G2P1L1	III	clear	-	-	-	-	NG																																	
359	2756	28	G2P1L1	III	clear	-	-	-	-	NG																																	
360	1956	23	Primi	II	clear	-	-	-	-	NG																																	
361	2367	21	Primi	III	clear	-	-	-	-	NG																																	
362	996	32	G2P1L1	III	clear	-	-	-	-	NG																																	
363	4180	30	Primi	II	turbid	-	-	-	-	NG																																	
364	3331	30	G2P1L1	II	clear	-	-	-	-	NG																																	
365	4179	34	G2P1L1	III	clear	-	-	-	-	NG																																	
366	1332	29	G2P1L1	III	clear	-	-	-	-	NG																																	
367	4178	29	G3P2L2	I	clear	+	-	+	+	Proteus mirabilis	+				+	+								R	S	R	S	S	R	R	R	R	S	R	S	S					
368	3412	20	Primi	II	clear	-	-	-	-	NG																																	
369	2376	27	G3P2L1	III	clear	-	-	-	-	NG																																	

370	4185	20	Primi	III	clear	+	-	-	-	NG																																		
371	3424	26	G2P1L1	I	clear	-	-	-	-	NG																																			
372	1211	20	Primi	III	clear	-	-	-	-	NG																																			
373	2975	23	Primi	III	turbid	-	-	-	-	NG																																			
374	2781	23	Primi	II	clear	-	-	-	-	NG																																			
375	4094	23	G2P1L1	II	clear	-	-	-	-	NG																																			
376	1698	25	G3P2L1	II	clear	-	-	+	-	NG																																			
377	8945	20	G2P1L1	I	clear	-	-	-	-	NG																																			
378	4187	24	G3P2L2	II	clear	-	-	-	-	NG																																			
379	4233	21	Primi	II	clear	-	-	-	-	NG																																			
380	3581	26	G2P1L1	I	clear	-	-	-	-	NG																																			
381	3623	23	Primi	I	clear	-	-	-	-	NG																																			
382	2891	31	G3P2L2	III	clear	-	-	-	-	NG																																			
383	2342	26	G2P1L1	II	clear	-	-	-	-	NG																																			
384	3355	20	Primi	I	clear	-	-	-	-	NG																																			
385	3983	24	Primi	II	clear	-	-	-	-	NG																																			
386	3128	22	G2P1L1	I	clear	-	-	-	-	NG																																			
387	3319	31	G2P1L1	II	clear	-	-	-	-	NG																																			
388	2183	29	Primi	III	clear	-	-	-	-	NG																																			
389	3390	31	G3P1L1A1	I	clear	+	+	+	+	Klebsiella pneumoniae							+						R	R	R	R	R	S	S	S	R	S	S	S	S			ESBL
390	4496	20	Primi	I	turbid	+	+	+	-	Escherichia coli		+	+										R	S	S	S	S	S	S	S	S	S	S	S	S			
391	3056	23	G2P1L1	II	clear	-	-	-	-	NG																																	
392	10293	31	G2P1L1	II	clear	+	-	-	-	NG																																			
393	4210	25	Primi	III	clear	-	-	-	-	NG																																			
394	3074	25	Primi	I	clear	-	-	-	-	NG																																			
395	4211	26	Primi	II	turbid	-	-	-	-	NG																																			
396	4217	31	G3P2L2	II	clear	+	+	+	+	Staphylococcus saprophyticus						+							S	S	S	S		S	S	R	R	S	R			S	S		
397	2299	27	Primi	III	clear	-	-	-	-	NG																																			
398	3457	19	Primi	II	clear	-	-	-	-	NG																																			
399	2199	18	Primi	I	clear	-	-	-	-	NG																																			
400	5318	23	G2P1L1	III	clear	-	-	-	+	NG																																			

401	3866	26	G2P1L1	II	clear	-	-	-	-	NG																													
402	3996	25	Primi	III	clear	-	+	+	+	Escherichia coli														S	S	R	S	S	S	R	R	S	S	S	S	S			
403	4658	22	Primi	III	clear	-	-	-	-	NG																													
404	3988	32	Primi	III	clear	-	-	-	-	NG																													
405	4661	26	G2P1L1	III	clear	-	-	-	-	NG																													
406	2951	25	Primi	II	clear	-	-	-	-	NG																													
407	3622	20	G2P1L1	III	clear	-	-	-	-	NG																													
408	4652	20	Primi	III	clear	-	-	-	-	NG																													
409	3990	30	G3P2L1	III	clear	-	-	-	-	NG																													
410	3654	27	G2P1L1	II	clear	-	-	-	-	NG																													
411	4653	18	Primi	I	clear	+	+	-	-	Escherichia coli				+	+	+								S	S	S	S	S	S	S	S	R	S	S	S	S			
412	4659	28	Primi	II	clear	-	-	-	-	NG																													
413	3639	20	Primi	III	clear	-	-	-	-	NG																													
414	4663	35	G3P2L2	II	clear	-	-	-	-	NG																													
415	4662	25	G2P1L1	III	clear	-	-	-	-	NG																													
416	2618	23	G2P1L1	I	clear	-	-	-	-	NG																													
417	9805	21	G2P1L1	I	clear	-	-	-	-	NG																													
418	1465	26	Primi	III	turbid	-	-	-	-	NG																													
419	4642	21	G3P0L0A2	II	turbid	-	-	-	-	NG																													
420	4562	27	Primi	III	clear	+	-	-	-	NG																													
421	4561	31	G2P1L1	I	clear	-	-	-	-	NG																													
422	4573	21	G2P0L0A1	I	clear	-	-	-	-	NG																													
423	4565	24	G2P1L1	II	clear	-	-	-	-	NG																													
424	9630	25	G2P1L1	II	clear	-	-	+	-	Klebsiella pneumoniae							+							R	R	R	S	S	R	S	R	S	R	S	S	S			
425	1430	20	Primi	II	clear	-	-	-	-	NG																													
426	6522	29	G3P2L2	III	clear	-	-	-	-	NG																													
427	4618	19	Primi	I	clear	-	-	-	-	NG																													
428	4412	25	G3P1L1A1	II	clear	-	-	-	-	NG																													
429	4700	21	Primi	I	clear	-	-	-	-	NG																													
430	4621	23	Primi	II	clear	-	-	-	-	NG																													
431	2561	25	G2P1L1	II	clear	+	+	-	+	Staphylococcus aureus	+					+			+	+	+	+	+	R	S	R	S		R	S	R	R	S	R			S	S	

432	4081	26	G3P1L1A1	III	clear	-	-	-	-	NG																																
433	1626	29	G3P2L2	III	clear	-	-	-	-	NG																																
434	3653	31	G2P1L1	II	clear	-	-	+	+	Escherichia coli	+												R	S	S	S	S	S	S	S	S	S	S	S	S	S				
435	4073	30	Primi	III	clear	-	-	-	-	NG																																
436	4629	28	G2P1L1	I	clear	-	-	-	+	NG																																
437	9686	22	Primi	I	clear	-	-	-	-	NG																																
438	4393	25	G2P1L1	II	clear	-	-	-	-	NG																																
439	3854	27	G2P1L1	II	clear	+	-	-	-	NG																																
440	4632	21	Primi	I	clear	-	-	-	-	NG																																
441	4576	19	Primi	I	clear	-	-	-	-	NG																																
442	4093	26	G2P1L1	II	clear	-	-	-	-	NG																																
443	3574	27	G3P2L2	II	clear	-	-	-	-	NG																																
444	2897	23	PRIMI	I	clear	-	-	+	-	NG																																
445	1492	20	Primi	I	turbid	-	-	-	-	NG																																
446	3207	21	Primi	III	clear	-	-	-	-	NG																																
447	3335	23	Primi	II	clear	+	+	+	-	Enterococcus faecalis	+												S	S				R	S	R	S	S	R			S				
448	2287	27	G2PILI	II	clear	-	-	-	-	NG																																
449	4512	24	G2PILI	III	clear	-	-	-	-	NG																																
450	1299	28	G3PILIAI	I	clear	-	-	-	+	NG																																
451	3277	25	PRIMI	II	clear	-	-	-	-	NG																																
452	2265	29	G3P2L2	II	clear	-	-	-	-	NG																																
453	3276	30	G4P2L2A1	III	clear	-	-	-	-	NG																																
454	5471	24	Primi	I	clear	-	-	-	-	NG																																
455	5291	28	G2P1L1	II	clear	-	-	-	+	NG																																
456	5342	26	G2P1L1	II	clear	-	-	-	-	NG																																
457	1099	27	G2P1L1	III	clear	-	-	-	-	NG																																
458	1567	23	Primi	II	clear	-	-	-	-	NG																																
459	5897	25	Primi	I	clear	-	-	-	-	NG																																
460	3492	23	Primi	I	clear	+	-	-	-	NG																																
461	3386	22	Primi	II	clear	-	-	-	-	NG																																
462	1906	25	G2P1L1	III	clear	-	-	-	-	NG																																

463	1789	32	G3P2L2	II	clear	-	-	-	-	NG																																	
464	1654	22	Primi	I	clear	-	-	-	-	NG																																	
465	3254	18	Primi	II	turbid	-	-	-	-	NG																																	
466	4467	21	Primi	III	clear	-	-	-	-	NG																																	
467	1556	21	Primi	III	turbid	-	-	-	-	NG																																	
468	1543	24	G2P1L1	I	clear	-	-	-	-	NG																																	
469	3330	20	Primi	II	clear	-	-	-	-	NG																																	
470	2431	23	G2P1L1	I	clear	-	-	-	-	NG																																	
471	4657	30	G3P2L2	III	clear	-	-	-	-	NG																																	
472	3338	27	G3P0L0A2	III	clear	-	-	-	-	NG																																	
473	1673	24	Primi	I	clear	-	-	-	-	NG																																	
474	1324	24	Primi	I	turbid	-	+	-	+	Klebsiella pneumoniae							+							R	R	S	S	S	S	R	S	S	R	R	S	S				
475	1783	24	G2P1L1	II	clear	+	-	-	-	NG																																	
476	2156	27	G2P1L1	II	clear	-	-	-	-	NG																																	
477	3578	28	G3P2L2	III	clear	-	-	-	-	NG																																	
478	3321	29	Primi	I	clear	-	-	-	-	NG																																	
479	5623	25	Primi	I	clear	-	-	-	-	NG																																	
480	1054	22	Primi	II	clear	-	-	-	-	NG																																	
481	1056	25	Primi	II	clear	-	-	-	-	NG																																	
482	1059	26	G2P1L1	III	clear	-	-	-	-	NG																																	
483	4499	27	G2P1L1	III	clear	-	-	-	-	NG																																	
484	2143	26	G2P1L1	III	clear	-	-	-	-	NG																																	
485	5498	28	G2P1L1	II	turbid	+	+	+	+	Proteus mirabilis	+		+											R	R	R	S	S	S	R	S	S	R	S	S	S				
486	4091	27	Primi	III	clear	-	-	-	-	NG																																	
487	3358	19	Primi	III	clear	-	-	-	-	NG																																	
488	2365	19	Primi	III	clear	-	-	-	-	NG																																	
489	2109	23	G2P0L0A1	I	clear	-	-	-	-	NG																																	
490	3256	27	G3P0L0A2	II	clear	-	-	-	-	NG																																	
491	2190	23	Primi	II	clear	-	-	-	-	NG																																	
492	3091	24	Primi	II	clear	-	-	-	-	NG																																	
493	5632	22	Primi	III	clear	+	-	-	-	NG																																	

494	3330	21	Primi	I	clear	-	-	-	-	NG																																	
495	2274	24	G2PILI	II	clear	-	-	+	-	NG																																	
496	3219	24	G2P1L1	I	clear	-	-	-	-	NG																																	
497	5318	27	Primi	II	turbid	+	+	+	+	Klebsiella pneumoniae							+							R	R	R	S	S	S	R	R	S	S	S	S	S				
498	2221	33	G3P1LIA1	III	clear	-	-	-	-	NG																																	
499	3980	22	Primi	II	clear	-	-	-	-	NG																																	
500	3465	23	Primi	I	clear	-	-	-	-	NG																																	
501	6574	32	G2P1L1	II	clear	-	-	-	-	NG																																	
502	6788	22	Primi	III	turbid	-	-	-	-	NG																																	
503	2280	28	G2P1L1	I	clear	-	-	-	-	NG																																	
504	6789	20	Primi	II	clear	-	-	-	-	NG																																	
505	6790	23	Primi	I	clear	-	+	+	+	Klebsiella pneumoniae						+	+							R	S	R	S	S	S	S	S	R	R	R	S	S				
506	6795	21	Primi	III	clear	-	-	-	-	NG																																	
507	2522	19	Primi	II	clear	-	+	-	-	Staphylococcus saprophyticus														R	S	S	S		S	S	S	R	S	S			S	S		
508	4388	25	G2P1L1	I	clear	-	-	-	-	NG																																	
509	5781	25	G2P1L1	I	clear	+	+	+	+	Klebsiella pneumoniae							+							R	R	R	S	S	S	S	R	S	S	S	S	S				
510	5558	19	Primi	I	clear	-	+	+	+	Escherichia coli				+										S	S	R	S	S	S	S	R	R	S	S	S	S				
511	4217	24	G2P1L1	III	clear	-	-	-	-	NG																																	
512	5670	28	G2P1L1	I	turbid	+	+	+	+	Escherichia coli	+													S	S	S	S	S	S	S	R	S	S	R	S	S				
513	5099	20	Primi	II	turbid	-	-	-	-	NG																																	
514	3195	20	Primi	II	clear	+	-	-	-	NG																																	
515	2108	20	G2P1L1	III	clear	-	-	-	-	NG																																	
516	10164	22	Primi	II	clear	-	-	-	-	NG																																	
517	6432	18	Primi	II	turbid	-	+	+	+	Escherichia coli		+												R	S	R	S	S	S	S	R	S	S	S	S	S				
518	6779	24	Primi	I	clear	-	-	-	-	NG																																	
519	9856	23	G2P1L1	III	clear	-	-	-	-	NG																																	
520	4320	18	Primi	I	turbid	+	-	+	+	Staphylococcus aureus	+					+			+	+	+	+	+	S	S	S	S		S	R	R	S	S	S			S	S		
521	5570	21	Primi	II	clear	-	-	-	-	NG																																	
522	5007	24	G2PILI	III	clear	-	-	-	-	NG																																	
523	4488	26	G2PILI	III	clear	-	-	-	-	NG																																	
524	10408	39	G3P0L0A2	III	clear	-	+	-	-	Enterococcus faecalis						+								R	S				S	S	S	S	S	R			S			

525	3242	18	Primi	I	clear	-	-	-	-	NG																													
526	6622	23	Primi	I	clear	-	-	-	-	NG																													
527	7890	20	Primi	II	clear	-	-	-	-	NG																													
528	4529	29	G2PILI	III	clear	-	-	-	-	NG																													
529	4522	27	G2PILI	II	clear	-	-	-	-	NG																													
530	3211	19	Primi	II	clear	-	-	-	-	NG																													
531	3399	21	Primi	I	clear	-	+	-	+	Enterococcus faecalis	+													R	R				S	R	R	R	S	S			S		
532	5679	20	Primi	I	clear	-	-	-	-	NG																													
533	1092	25	G2PILI	II	clear	-	-	-	-	NG																													
534	10431	18	Primi	I	turbid	-	-	-	-	NG																													
535	4567	27	G2PILI	II	clear	-	-	-	-	NG																													
536	1299	24	Primi	III	clear	-	-	-	-	NG																													
537	4490	19	Primi	I	clear	-	-	-	-	NG																													
538	3597	20	Primi	II	turbid	+	+	+	+	Staphylococcus saprophyticus														S	S	R	S		S	S	S	S	S	S			S	S	
539	2188	23	Primi	II	clear	-	-	+	-	NG																													
540	4869	21	Primi	II	clear	-	-	-	-	NG																													
541	12309	25	G3P2L2	III	clear	-	-	+	-	Klebsiella pneumoniae							+							R	S	S	S	S	S	S	S	R	R	S	S	S			
542	23450	37	G2PILI	II	clear	-	-	-	-	NG																													
543	35621	26	G2PILI	I	clear	+	-	-	-	NG																													
544	3339	22	Primi	II	clear	-	-	-	-	NG																													
545	3677	21	Primi	II	clear	-	-	-	-	NG																													
546	3680	35	G3P2L2	III	clear	-	-	-	+	NG																													
547	3677	26	Primi	I	clear	-	-	-	-	NG																													
548	5682	25	G2PILI	II	clear	-	-	-	-	NG																													
549	23488	25	G2PILI	I	turbid	+	+	+	+	Escherichia coli														S	S	R	S	S	R	S	S	R	S	R	S	S			ESBL
550	56799	25	Primi	II	clear	-	-	-	-	NG																													
551	1785	21	Primi	III	turbid	-	-	-	-	NG																													
552	4168	18	Primi	I	turbid	-	-	-	-	NG																													
553	4359	20	Primi	II	turbid	+	+	+	+	Escherichia coli	+			+										S	S	S	S	S	S	S	S	S	S	R	S	S			
554	5428	21	G2PILI	III	clear	-	-	-	-	NG																													
555	4677	22	Primi	I	clear	-	-	-	-	NG																													

556	5536	25	Primi	II	clear	-	-	-	-	NG																																		
557	5271	26	G2PILI	I	clear	-	-	-	-	NG																																			
558	5672	20	Primi	III	clear	-	-	-	-	NG																																			
559	43109	23	G3P0L0A2	I	clear	+	+	+	+	Escherichia coli													S	S	S	S	S	S	R	R	S	S	S	S	S							
560	3579	20	Primi	I	clear	-	-	-	-	NG																																		
561	4486	19	Primi	II	clear	-	-	-	-	NG																																		
562	2409	21	G2PILI	II	clear	-	-	-	-	NG																																		
563	5334	27	G2PILI	III	clear	-	-	-	-	NG																																		
564	5431	30	G2P0L0A1	I	clear	-	-	-	-	NG																																		
565	4828	30	G2PILI	II	clear	-	-	-	-	NG																																		
566	5532	28	G2PILI	II	turbid	-	-	-	-	NG																																		
567	5531	20	Primi	I	clear	-	-	-	+	Staphylococcus aureus	+								+	+	+	+	+	S	S	R	R		S	S	R	R	S	R			S	S		
568	5530	27	Primi	II	turbid	-	-	-	-	NG																																		
569	23688	25	Primi	I	turbid	+	-	-	-	NG																																		
570	5529	26	G2PILI	I	turbid	-	-	-	-	NG																																		
571	5528	22	Primi	II	clear	-	+	-	-	NG																																		
572	3004	25	Primi	II	clear	-	+	+	+	Klebsiella pneumoniae							+						S	S	S	S	S	R	S	R	S	S	R	S	S							
573	3012	23	G2PILI	III	clear	-	-	-	-	NG																																		
574	3197	22	Primi	II	clear	-	-	-	-	NG																																		
575	11225	20	Primi	I	clear	-	-	-	-	NG																																		
576	1209	19	Primi	II	clear	-	-	-	-	NG																																		
577	2303	19	Primi	I	turbid	+	-	+	-	NG																																		
578	5262	18	Primi	I	turbid	-	-	-	-	NG																																		
579	4132	23	Primi	III	clear	-	-	-	-	NG																																		
580	18873	23	G2PILI	III	clear	-	-	-	-	NG																																		
581	5397	25	G2PILI	II	clear	-	-	-	-	NG																																		
582	5505	27	G3P2L2	II	clear	-	-	-	-	NG																																		
583	11339	21	Primi	III	clear	-	-	-	-	NG																																		
584	11335	20	Primi	II	clear	+	+	+	+	Enterococcus faecalis													S	S				S	S	S	R	S	R			S		
585	11224	18	Primi	I	clear	-	-	-	-	NG																																		
586	11340	25	G2PILI	I	clear	-	-	-	-	NG																																		

587	5791	23	Primi	I	clear	-	-	-	-	NG																																
588	47901	27	Primi	III	clear	-	-	-	-	NG																																
589	5132	29	G2P0L0A1	I	clear	-	-	-	-	NG																																
590	4030	30	G3P2L2	I	clear	-	+	+	-	Escherichia coli	+	+												R	S	R	S	S	S	S	S	S	R	S	S	S				
591	11227	31	Primi	II	turbid	-	-	-	-	NG																																
592	5512	20	Primi	I	clear	-	-	-	-	NG																																
593	5516	19	Primi	I	clear	-	-	-	-	NG																																
594	6037	25	G2PILI	II	clear	-	-	-	-	NG																																
595	2292	26	G2PILI	III	clear	-	+	+	+	Escherichia coli					+									S	S	S	S	S	R	S	R	S	S	S	S	S				
596	2217	23	G2PILI	I	clear	-	-	-	-	NG																																
597	5032	22	Primi	I	clear	-	-	-	-	NG																																
598	23864	20	Primi	I	turbid	-	-	-	+	NG																																
599	4684	30	G2PILI	I	clear	-	-	-	-	NG																																
600	5519	19	Primi	I	turbid	+	+	+	+	Klebsiella pneumoniae							+							R	R	R	S	S	S	S	S	R	S	R	S	S				
601	5809	23	Primi	II	clear	-	-	-	-	NG																																
602	5177	27	Primi	II	clear	-	-	-	-	NG																																
603	15690	24	G2PILI	III	clear	-	-	-	-	NG																																
604	1451	26	G3P2L2	III	clear	-	-	-	-	NG																																
605	5518	20	Primi	II	clear	-	-	-	-	NG																																
606	3036	21	Primi	II	clear	-	-	-	-	NG																																
607	6982	23	Primi	I	clear	-	-	-	-	NG																																
608	11464	25	Primi	I	clear	-	-	-	-	NG																																
609	11458	27	G2PILI	III	clear	-	-	-	-	NG																																
610	5668	22	Primi	II	turbid	-	-	-	+	NG																																
611	20981	29	G3P2L2	II	clear	-	-	-	-	NG																																
612	1779	21	Primi	I	turbid	-	-	-	-	NG																																
613	1256	20	Primi	I	clear	-	-	-	-	NG																																
614	1890	21	G2PILI	II	clear	-	+	-	+	Staphylococcus saprophyticus														R	S	S	R		S	S	R	S	S	S			S	S		
615	3299	23	Primi	I	clear	-	-	-	-	NG																																
616	5541	25	G2PILI	I	turbid	-	+	+	+	Escherichia coli			+			+								S	S	S	S	S	S	S	S	R	S	S	S	S				
617	5839	24	G3P0L0A2	I	clear	-	-	-	-	NG																																

618	21090	21	Primi	II	clear	-	-	-	-	NG																																		
619	46732	22	Primi	II	clear	-	-	-	-	NG																																			
620	24581	28	G2PILI	I	clear	-	-	-	-	NG																																			
621	27903	24	Primi	III	clear	-	-	-	-	NG																																			
622	22675	19	Primi	I	turbid	-	+	-	-	Staphylococcus aureus						+			+	+	+	+	+	S	S	R	R		S	S	S	S	S	S			S	S					
623	8432	23	G2PILI	II	clear	-	-	-	-	NG																																			
624	5742	32	G2PILI	I	clear	-	-	-	-	NG																																			
625	3215	19	Primi	II	clear	-	-	-	-	NG																																			
626	22210	23	Primi	II	clear	-	+	+	+	Escherichia coli													R	R	R	S	S	S	S	R	R	S	R	S	S							
627	1639	29	G3PIL2	I	clear	-	+	+	+	Escherichia coli													R	S	S	S	S	S	S	S	S	S	S	S	S							
628	14690	27	G2P1L1	III	clear	-	-	-	-	NG																																			
629	11488	24	Primi	I	clear	-	-	-	-	NG																																			
630	11505	29	G2PILI	I	clear	-	+	-	+	Staphylococcus aureus	+								+	+	+	+	+	S	S	S	S		R	S	R	R	S	S			S	S					
631	3661	28	Primi	III	clear	-	-	-	-	NG																																			
632	21998	20	Primi	III	turbid	-	-	+	-	Klebsiella pneumoniae							+							R	R	R	S	S	S	S	S	R	S	S	S	S							
633	2599	32	G2PILI	II	clear	-	-	-	-	NG																																			
634	11506	24	Primi	I	turbid	-	-	-	-	NG																																			
635	54399	23	Primi	I	clear	-	-	-	-	NG																																			
636	32100	21	Primi	II	clear	-	-	-	-	NG																																			
637	7634	22	Primi	II	clear	-	-	-	-	NG																																			
638	1991	25	Primi	III	clear	-	-	-	-	NG																																			
639	5392	21	G2PILI	II	clear	-	-	-	-	NG																																			
640	3714	27	G2PIL1	II	clear	-	-	-	-	NG																																			
641	5285	27	Primi	III	clear	-	-	-	-	NG																																			
642	5490	29	G2P0L0A1	I	clear	-	-	-	-	NG																																			
643	21790	30	G2PILI	I	clear	-	-	-	-	NG																																			
644	4329	22	Primi	I	clear	-	-	-	-	NG																																			
645	4644	35	G3PILIA1	II	turbid	-	-	-	-	NG																																			
646	2155	19	Primi	III	clear	-	-	-	-	NG																																			
647	2380	19	Primi	I	clear	-	-	-	-	NG																																			
648	2161	24	G2PILI	II	turbid	-	-	-	-	NG																																			

649	2700	20	Primi	II	turbid	-	+	+	-	Escherichia coli				+										R	S	S	S	S	S	S	R	S	S	R	S	S			S	S	
650	5629	24	Primi	I	clear	-	-	-	-	NG																																
651	11076	35	G3PILIA1	III	clear	-	-	-	-	NG																																
652	22109	23	G2PILI	II	clear	-	-	-	-	NG																																
653	2196	32	G2PILI	III	clear	-	-	-	-	NG																																
654	2387	26	G2P1L1	II	clear	-	-	-	-	NG																																
655	10902	30	G2P0L0A1	I	clear	-	-	-	-	NG																																
656	5620	24	G2PILI	II	clear	-	+	+	+	Staphylococcus saprophyticus														S	S	S	S		R	S	S	R	S	S			S	S	
657	3218	23	Primi	I	clear	-	-	-	-	NG																																
658	2166	23	Primi	I	clear	-	-	-	-	NG																																
659	1E+05	21	Primi	I	clear	-	-	-	-	NG																																
660	2136	21	Primi	I	turbid	+	+	+	+	Escherichia coli	+													S	S	R	S	S	S	S	S	S	S	R	S	S					
661	6781	22	Primi	I	clear	-	-	-	-	NG																																
662	3491	23	G2P1L1	III	clear	-	-	-	-	NG																																
663	21891	32	G2P1L1	I	clear	-	-	-	-	NG																																
664	5427	25	Primi	I	clear	-	-	-	-	NG																																
665	9563	20	Primi	I	clear	-	-	-	-	NG																																
666	3999	19	Primi	I	clear	-	-	-	-	NG																																
667	10524	22	G2P1L1	II	clear	-	-	-	+	NG																																
668	3288	26	G2P1L1	II	turbid	-	-	-	-	NG																																
669	8138	35	G3P2L2	II	clear	-	-	-	-	NG																																
670	6549	27	Primi	I	turbid	+	+	+	+	Escherichia coli														R	R	S	S	S	S	R	R	R	S	S	S	S					
671	2556	31	G2P1L1	III	clear	-	-	-	-	NG																																
672	2275	21	G2P1L1	I	clear	-	-	-	-	NG																																
673	2381	29	G2P1L1	II	clear	-	-	-	-	NG																																
674	10978	21	Primi	I	clear	-	-	-	-	NG																																
675	56546	35	G2P1L1	III	turbid	-	-	-	-	NG																																
676	10918	18	Primi	I	clear	-	-	-	-	NG																																
677	5364	27	G2P1L1	II	clear	-	+	-	-	Staphylococcus aureus							+		+	+	+	+	+	S	S	R	S		S	S	S	R	S	R			S	S	
678	10935	30	G2P1L1	III	clear	-	-	-	-	NG																																
679	5531	24	Primi	II	clear	-	-	-	-	NG																																

680	6459	26	Primi	I	clear	-	-	-	-	NG																																	
681	43210	18	Primi	I	clear	-	-	-	-	NG																																	
682	25678	20	Primi	I	clear	-	-	-	-	NG																																	
683	10763	30	G2P0L0A1	II	clear	-	-	-	-	NG																																	
684	4068	19	Primi	I	clear	-	-	-	-	NG																																	
685	1580	24	Primi	III	clear	-	-	-	-	NG																																	
686	4551	21	Primi	II	clear	-	-	-	-	NG																																	
687	4375	29	G2P1L1	I	clear	-	-	-	-	NG																																	
688	3256	26	Primi	III	clear	-	-	-	-	NG																																	
689	4218	35	G2P1L1	III	clear	-	-	-	-	NG																																	
690	3277	28	G2P1L1	III	clear	-	-	-	-	NG																																	
691	3329	21	Primi	I	turbid	-	+	+	+	Staphylococcus saprophyticus													R	S	R	S		S	R	S	S	S	R			S	S		
692	3571	27	G2P1L1	II	clear	-	-	-	-	NG																																	
693	6788	30	G2P1L1	III	clear	-	-	-	-	NG																																	
694	3357	29	G2P1L1	I	clear	-	-	-	-	NG																																	
695	2841	26	Primi	II	clear	-	-	-	-	NG																																	
696	45681	22	Primi	III	clear	-	-	-	-	NG																																	
697	32199	28	Primi	I	clear	-	-	-	-	NG																																	
698	2188	22	Primi	I	turbid	-	-	-	-	NG																																	
699	34660	25	G2P1L1	II	clear	-	-	-	-	NG																																	
700	3592	35	G3PILIA1	I	clear	-	-	-	-	NG																																	
701	4255	32	G3P2L2	III	turbid	+	-	+	+	Escherichia coli													R	S	S	R	R	S	S	S	S	S	S	S	S				
702	6572	24	Primi	I	turbid	-	-	-	-	NG																																	
703	2190	35	G2P1L1	I	clear	-	-	-	-	NG																																	
704	1E+05	37	G2P1L1	I	clear	-	-	-	-	NG																																	
705	32188	20	Primi	III	clear	-	-	-	-	NG																																	
706	1E+05	27	G2P1L1	I	clear	-	-	-	-	NG																																	
707	1E+05	30	G2P1L1	II	clear	-	-	-	-	NG																																	
708	11634	35	G2P1L1	III	clear	-	-	-	-	NG																																	
709	23756	30	G2P1L1	III	turbid	-	-	-	-	NG																																	
710	17890	28	G2P1L1	II	clear	-	-	-	-	NG																																	

711	12645	22	Primi	I	clear	-	-	-	-	NG																																
712	4209	25	Primi	I	clear	-	-	-	-	NG																																
713	5281	30	G2P1L1	II	clear	-	-	-	-	NG																																
714	2783	33	G3P2L1A1	III	clear	+	+	-	-	Escherichia coli				+	+									R	S	R	S	S	R	S	R	S	S	S	S	S					
715	45779	28	G2P1L1	I	clear	-	-	-	-	NG																																
716	37654	20	Primi	I	clear	-	-	+	+	Escherichia coli														S	S	S	S	S	S	S	R	R	S	S	S	S					
717	5468	30	G2P0L0A1	III	clear	-	-	-	-	NG																																
718	11961	31	G3P2L2	II	clear	-	-	-	-	NG																																
719	2764	29	G2P1L1	I	clear	-	-	-	-	NG																																
720	9871	24	Primi	I	clear	-	-	-	-	NG																																
721	3178	34	G2P1L1	III	turbid	-	-	-	-	NG																																
722	10818	32	G2P1L1	I	clear	-	+	+	-	Staphylococcus saprophyticus														R	S	R	S		S	R	S	S	S	S			S	S		
723	5253	27	G2P1L1	II	clear	-	-	-	-	NG																																
724	3577	21	Primi	II	clear	-	-	-	-	NG																																
725	3298	33	G2P1L1	III	clear	-	-	-	-	NG																																
726	1588	25	Primi	I	clear	-	-	-	-	NG																																
727	6633	22	Primi	II	clear	-	-	-	-	NG																																
728	35790	21	Primi	I	clear	-	-	-	-	NG																																
729	36712	22	Primi	III	clear	-	-	-	-	NG																																
730	4903	23	G2P1L1	I	clear	-	-	-	-	NG																																
731	11995	38	G3P1L1AI	I	clear	-	-	-	-	NG																																
732	54673	30	G2P1L1	II	clear	-	-	-	-	NG																																
733	12129	26	G2P1L1	II	clear	-	-	-	-	NG																																
734	36475	28	G2P1L1	III	clear	-	-	-	-	NG																																
735	23456	25	Primi	II	turbid	-	-	+	-	NG																																
736	36361	24	Primi	I	turbid	-	-	-	-	NG																																
737	5684	19	Primi	I	clear	-	+	+	-	Klebsiella pneumoniae							+							R	S	S	S	S	S	S	R	R	R	R	S	S					
738	24576	26	Primi	I	clear	-	-	-	-	NG																																
739	2145	22	Primi	I	clear	-	-	-	-	NG																																
740	34567	21	Primi	III	clear	-	-	-	-	NG																																
741	24318	33	G2P1L1	II	turbid	-	-	-	-	NG																																

742	23458	20	Primi	II	clear	-	-	-	-	NG																																
743	9792	29	G2P1L1	III	turbid	-	-	-	-	NG																																
744	1222	21	Primi	I	clear	-	-	-	-	NG																																
745	3298	20	Primi	I	turbid	-	+	+	+	Staphylococcus aureus							+		+	+	+	+	+	S	S	R	S		S	R	R	S	R	S			S	S			
746	6482	27	G2P1L1	III	clear	-	-	-	-	NG																																
747	3645	24	Primi	II	clear	-	-	-	-	NG																																
748	12748	22	Primi	I	clear	-	-	-	-	NG																																
749	5355	30	Primi	III	clear	-	-	-	-	NG																																
750	3477	29	Primi	I	clear	-	-	-	-	NG																																
751	65430	27	G2P1L1	III	turbid	-	-	-	-	NG																																
752	21980	35	G2P1L1	II	clear	-	-	-	-	NG																																
753	5791	33	G2P1L1	II	clear	-	-	-	-	NG																																
754	23549	28	G2P1L1	I	clear	-	-	-	-	NG																																
755	5619	29	G2P0L0A1	I	clear	-	-	-	-	NG																																
756	3974	19	Primi	I	clear	-	-	-	-	NG																																
757	5338	23	Primi	II	clear	-	-	-	-	NG																																
758	2748	20	Primi	I	clear	-	-	-	-	NG																																
759	12346	20	Primi	I	clear	-	-	-	-	NG																																
760	32648	23	G2P1L1	III	clear	-	-	-	-	NG																																
761	64352	25	G2P1L1	II	turbid	+	+	+	+	Escherichia coli			+										R	S	S	S	S	S	S	S	S	R	S	S	S				
762	32409	20	Primi	I	clear	-	-	-	+	NG																																
763	4441	26	Primi	III	clear	-	-	-	-	NG																																
764	5876	26	G2P1L1	II	clear	-	+	-	-	NG																																
765	53521	33	G2P1L1	II	clear	-	-	-	-	NG																																
766	3210	22	Primi	I	clear	-	-	-	-	NG																																
767	54091	24	Primi	II	clear	-	-	-	-	NG																																
768	23898	30	G2P1L1	III	turbid	-	-	-	-	NG																																
769	6971	30	G2P1L1	III	clear	-	-	-	-	NG																																
770	19830	24	Primi	II	clear	-	-	-	-	NG																																
771	13221	30	G2P1L1	I	clear	-	-	-	-	NG																																
772	5898	29	G3P0L0A2	I	clear	-	-	-	-	NG																																

773	5891	21	Primi	II	clear	-	-	-	-	NG																																	
774	7639	20	Primi	III	turbid	-	-	-	-	NG																																	
775	12773	29	G2P1L1	II	clear	-	-	-	-	NG																																	
776	5778	23	Primi	III	clear	-	-	-	-	NG																																	
777	23556	24	Primi	I	clear	-	-	-	-	NG																																	
778	23477	21	Primi	I	clear	-	-	-	-	NG																																	
779	21987	21	Primi	I	clear	-	-	-	-	NG																																	
780	34661	25	Primi	II	clear	-	-	-	-	NG																																	
781	3652	25	Primi	III	clear	-	-	-	-	NG																																	
782	32908	30	G2P1L1	II	turbid	-	-	-	-	NG																																	
783	3115	23	Primi	II	clear	-	-	-	-	NG																																	
784	4556	25	Primi	III	clear	-	-	-	-	NG																																	
785	2233	23	Primi	II	clear	+	+	+	+	Escherichia coli	+				+									R	R	S	S	S	S	S	R	S	S	S	S	S					
786	5571	39	G3P1L1AI	I	turbid	-	-	-	-	NG																																	
787	5011	36	G2P1L1	I	clear	-	-	-	-	NG																																	
788	4590	34	G2P1L1	III	clear	-	-	-	-	NG																																	
789	1890	23	Primi	I	clear	-	-	-	-	NG																																	
790	21775	24	Primi	II	clear	+	+	+	+	Escherichia coli					+									R	R	R	S	S	S	S	S	S	S	R	S	S					
791	962	32	G2P1L1	I	clear	-	-	-	-	NG																																	
792	12240	31	G3P2L2	I	clear	-	-	+	-	NG																																	
793	1778	23	Primi	III	clear	-	-	-	-	NG																																	
794	12662	21	G2P1L1	II	clear	-	-	-	-	NG																																	
795	12389	25	G2P1L1	III	clear	-	-	-	-	NG																																	
796	6523	19	Primi	II	turbid	-	-	-	-	NG																																	
797	5403	21	Primi	I	turbid	-	-	-	-	NG																																	
798	73420	29	G2P1L1	I	clear	-	-	-	-	NG																																	
799	2563	24	Primi	III	clear	-	-	-	-	NG																																	
800	7770	33	G2P1L1	II	clear	-	-	-	-	NG																																	
801	4714	23	Primi	I	clear	-	-	-	-	NG																																	
802	2506	22	Primi	II	clear	-	-	-	-	NG																																	
803	4935	26	G2P1L1	III	clear	-	-	-	-	NG																																	

804	23465	22	Primi	I	clear	-	-	-	-	NG																																
805	79091	31	G2P1L1	II	turbid	-	-	+	-	Escherichia coli	+														R	S	R	S	S	S	S	S	S	S	S	S	S				
806	3278	21	Primi	I	clear	+	-	-	-	NG																																
807	76412	28	Primi	I	clear	-	-	-	-	NG																																
808	239	34	G2P1L1	II	clear	-	-	-	-	NG																																
809	4681	28	G2P1L1	I	clear	-	-	-	-	NG																																
810	23564	27	G2P1L1	II	clear	-	-	-	-	NG																																
811	66721	20	Primi	II	clear	-	-	-	-	NG																																
812	78192	19	Primi	III	clear	-	-	-	-	NG																																
813	2789	22	Primi	II	clear	-	-	-	-	NG																																
814	7522	23	Primi	I	clear	-	-	-	-	NG																																
815	8218	31	G2P1L1	III	clear	-	-	-	-	NG																																
816	8374	21	Primi	I	clear	-	-	-	-	NG																																
817	7513	25	Primi	II	turbid	-	-	-	-	NG																																
818	7310	32	G2P1L1	III	clear	-	-	-	-	NG																																
819	1224	26	Primi	II	turbid	-	-	-	-	NG																																
820	5757	29	G2P1L1	III	clear	-	-	-	-	NG																																
821	2830	28	G2P1L1	II	clear	-	-	-	-	NG																																
822	13828	21	Primi	I	turbid	-	-	-	-	NG																																
823	1012	24	Primi	II	clear	-	-	-	-	NG																																
824	3199	23	Primi	I	clear	-	-	-	-	NG																																
825	4312	22	Primi	I	clear	-	-	-	-	NG																																
826	5400	24	Primi	II	clear	-	-	-	-	NG																																
827	4165	22	Primi	III	clear	-	-	-	-	NG																																
828	3295	26	G2P1L1	II	clear	-	-	-	-	NG																																
829	1454	18	Primi	I	clear	-	-	-	-	NG																																
830	6123	20	Primi	I	clear	-	-	-	-	NG																																
831	3336	26	G2P1L1	III	turbid	-	-	-	-	NG																																
832	1544	28	G2P1L1	II	turbid	-	-	-	-	NG																																
833	8611	25	Primi	I	clear	-	+	+	+	Staphylococcus saprophyticus															S	S	S	S		R	S	R	R	S	S			S	S	
834	11365	20	Primi	II	clear	-	-	-	-	NG																																

835	8641	25	G2P1L1	III	clear	-	-	-	-	NG																																
836	545	23	Primi	I	clear	-	+	-	+	Enterococcus faecalis	+					+								R	R				R	S	S	S	S	S			S		
837	3910	20	Primi	I	clear	-	-	-	+	NG																																
838	1999	29	G3P2L2	II	clear	-	-	-	-	NG																																
839	2108	28	G2P1L1	II	clear	-	-	-	-	NG																																
840	6540	28	G2P1L1	II	clear	+	+	+	-	Escherichia coli			+											R	S	R	S	S	S	S	R	S	S	S	S	S					
841	2112	27	G2P1L1	III	clear	-	-	-	-	NG																																
842	4481	20	Primi	II	clear	-	-	-	+	NG																																
843	5019	22	G2P1L1	I	clear	-	-	-	-	NG																																
844	4671	37	G3P1L1A1	III	clear	-	-	-	-	NG																																
845	3079	20	G2P1L1	I	clear	-	+	+	-	Escherichia coli														R	R	S	S	S	S	S	S	S	R	S	S	S					
846	3339	26	G2P1L1	II	clear	-	-	-	-	NG																																
847	2873	24	Primi	I	clear	-	-	-	-	NG																																
848	2248	20	Primi	III	clear	-	-	-	-	NG																																
849	3264	21	Primi	II	clear	-	-	-	-	NG																																
850	208	27	G2P1L1	II	clear	-	-	-	-	NG																																
851	42176	22	G2P1L1	II	clear	-	+	+	+	Escherichia coli						+								S	S	R	S	S	S	R	R	S	S	R	S	S					
852	4015	22	Primi	III	clear	-	-	-	-	NG																																
853	581	23	Primi	II	clear	-	-	-	-	NG																																
854	645	19	Primi	I	clear	-	-	-	-	NG																																
855	1771	26	G2P1L1	II	clear	-	-	-	-	NG																																
856	2256	38	G2P1L1	I	turbid	-	+	+	-	Escherichia coli														R	S	R	S	S	S	S	R	S	S	S	S	S					
857	2512	28	G2P1L1	II	clear	-	-	-	-	NG																																
858	1437	26	Primi	III	clear	-	-	-	-	NG																																
859	658	23	Primi	II	clear	-	-	-	-	NG																																
860	5701	24	G2P1L1	I	clear	-	-	-	-	NG																																
861	3183	25	G2P1L1	III	clear	-	-	+	+	Escherichia coli		+	+											R	S	S	S	S	R	S	S	R	R	S	S	S					
862	3452	18	Primi	I	clear	-	-	-	-	NG																																
863	2670	22	Primi	III	clear	-	-	-	-	NG																																
864	4340	27	G2P1L1	III	clear	+	+	+	-	Escherichia coli	+	+												S	S	S	S	S	S	S	R	S	R	S	S	S					
865	725	36	G2P1L1	II	clear	-	-	-	-	NG																																

866	16963	28	G3P2L2	II	turbid	-	-	-	-	NG																																		
867	3680	23	Primi	I	clear	-	-	-	-	NG																																		
868	2801	21	Primi	II	clear	-	-	-	-	NG																																		
869	7632	20	Primi	II	turbid	-	+	+	+	Escherichia coli														R	S	R	S	S	R	S	R	R	R	S	S	S					
870	12673	23	G3P0L0A2	I	clear	-	+	+	+	Escherichia coli														R	S	R	S	S	R	S	S	R	S	S	S	S					
871	2824	23	G2P1L1	I	clear	-	-	-	-	NG																																		
872	65263	22	G2P1L1	I	clear	-	-	+	+	Escherichia coli			+											R	R	S	S	S	R	S	S	S	S	R	S	S					
873	15254	33	G3P1L1A1	II	clear	-	-	+	-	Escherichia coli	+			+										R	S	R	R	R	S	S	R	S	S	S	S	S					ESBL
874	2812	22	Primi	III	clear	-	-	-	-	NG																																		
875	6530	24	G2P1L1	III	clear	-	-	-	-	NG																																		
876	5553	22	Primi	III	clear	-	-	-	-	NG																																		
877	6253	20	Primi	I	turbid	-	+	-	+	Escherichia coli														R	R	S	S	S	S	S	S	R	S	S	S	S					
878	3554	26	G2P1L1	II	clear	-	-	-	-	NG																																		
879	5122	22	Primi	I	clear	-	-	-	-	NG																																		
880	12467	21	G2P1L1	II	clear	-	-	-	-	NG																																		
881	12466	18	Primi	I	clear	-	-	-	-	NG																																		
882	5612	19	Primi	III	clear	-	-	-	-	NG																																		
883	43109	22	Primi	I	clear	-	+	-	-	Escherichia coli														R	R	R	S	S	S	S	S	S	R	R	S	S					
884	5342	23	Primi	I	clear	-	+	-	-	Staphylococcus aureus							+		+	+	+	+	+	S	S	S	R		S	S	R	S	S	R			S	S			
885	6263	25	G2P1L1	II	turbid	-	-	-	-	NG																																		
886	12679	27	G2P1L1	II	clear	-	-	-	-	NG																																		
887	12523	34	G2P1L1	III	clear	-	-	-	-	NG																																		
888	5231	27	G2P1L1	III	clear	-	+	+	+	Enterococcus faecalis	+													R	S				S	S	S	S	S	R			S				
889	5243	20	Primi	I	clear	-	-	-	-	NG																																		
890	7614	25	G2P1L1	II	turbid	-	-	-	-	NG																																		
891	62671	34	G2P1L2	I	clear	-	+	+	+	Escherichia coli														S	S	S	S	S	S	S	S	S	R	S	S	S					
892	2341	19	Primi	II	clear	-	+	-	-	NG																																		
893	4526	23	G2P1L1	II	clear	-	-	-	-	NG																																		
894	19593	22	Primi	I	clear	-	-	-	-	NG																																		
895	5423	27	G2P1L1	III	clear	-	+	+	-	Staphylococcus saprophyticus														R	S	S	R		S	S	S	S	R	R			S	S			
896	12611	18	Primi	I	clear	-	-	-	-	NG																																		

897	1527	25	G2P1L1	I	clear	-	-	-	-	NG																																			
898	1E+05	30	G3P1L1A1	III	turbid	-	-	-	-	NG																																			
899	44321	20	Primi	I	turbid	-	+	-	-	Staphylococcus aureus							+		+	+	+	+	+	S	S	S	S		R	S	S	R	S	R			S	S							
900	23410	37	G2P1L1	II	clear	-	-	-	-	NG																																			
901	4531	28	G2P1L1	III	clear	-	-	-	-	Enterococcus faecalis				+										R	R				S	S	S	R	R	S			S								
902	6034	19	Primi	I	clear	-	-	-	-	NG																																			
903	2432	24	Primi	III	clear	-	-	-	-	NG																																			
904	3258	30	G2P1L1	II	clear	-	-	-	-	NG																																			
905	5309	20	Primi	II	clear	-	-	-	-	NG																																			
906	23561	19	Primi	I	clear	-	-	-	-	NG																																			
907	5364	21	Primi	III	clear	-	+	+	+	Staphylococcus aureus						+			+	+	+	+	+	R	R	R	S		S	S	S	R	R	S			S	S							
908	12654	35	G2P1L1	II	turbid	-	-	-	-	NG																																			
909	2804	22	Primi	III	clear	-	-	-	-	NG																																			
910	2940	24	G2P1L1	I	clear	-	-	-	-	NG																																			
911	3032	31	G2P0L0A1	II	clear	-	-	-	-	NG																																			
912	2588	20	Primi	III	clear	-	-	-	-	NG																																			
913	19375	20	Primi	II	clear	-	-	-	-	NG																																			
914	4172	19	Primi	I	clear	-	-	-	-	NG																																			
915	3173	22	Primi	III	clear	-	-	-	-	NG																																			
916	62366	20	Primi	I	clear	-	+	+	+	Enterococcus faecalis														R	S				R	R	R	R	S	S			S								
917	3035	20	Primi	III	clear	-	-	-	-	NG																																			
918	2335	20	Primi	II	clear	-	-	-	-	NG																																			
919	4390	25	G2P1L1	I	turbid	-	-	-	-	NG																																			
920	3076	29	G3P1L1A1	I	clear	-	-	-	-	NG																																			
921	3372	23	Primi	III	clear	-	-	-	-	NG																																			
922	15184	18	Primi	II	turbid	-	-	-	-	NG																																			
923	2614	28	G2P1L1	III	turbid	-	-	-	-	NG																																			
924	3139	24	Primi	I	clear	-	-	-	-	NG																																			
925	648	25	Primi	III	clear	-	-	-	-	NG																																			
926	21889	21	Primi	II	clear	-	-	-	-	NG																																			
927	5537	24	G2P1L1	III	clear	-	-	-	-	NG																																			

928	4591	20	Primi	I	clear	-	-	-	-	NG																																	
929	1031	32	G2P1L1	II	clear	-	-	-	-	NG																																	
930	3527	23	Primi	II	turbid	-	-	-	-	NG																																	
931	1028	25	G2P1L1	III	clear	-	-	-	-	NG																																	
932	23810	30	G2P1L1	III	clear	-	-	-	-	NG																																	
933	3535	21	Primi	II	turbid	-	-	-	-	NG																																	
934	502	20	Primi	II	clear	-	-	-	-	NG																																	
935	3633	24	Primi	III	clear	-	-	-	-	NG																																	
936	2144	25	Primi	II	clear	-	-	-	-	NG																																	
937	3459	18	Primi	III	clear	-	-	-	-	NG																																	
938	3341	33	G2P1L1	II	turbid	-	-	-	-	NG																																	
939	934	18	Primi	I	clear	-	-	-	-	NG																																	
940	1129	35	G2P1L1	III	turbid	-	-	-	-	NG																																	
941	2625	21	Primi	I	clear	-	-	-	-	NG																																	
942	3357	18	Primi	I	clear	-	-	-	-	NG																																	
943	2478	26	G2P1L1	II	clear	-	-	-	-	NG																																	
944	1214	23	Primi	II	clear	-	-	-	-	NG																																	
945	3741	22	Primi	I	turbid	-	-	-	-	NG																																	
946	25975	22	Primi	II	clear	-	-	-	-	NG																																	
947	3914	29	G2P1L1	I	turbid	-	-	-	-	NG																																	
948	2711	23	Primi	III	clear	-	-	-	-	NG																																	
949	3944	20	Primi	I	clear	-	-	-	-	NG																																	
950	3667	26	G2p1L1	II	turbid	+	+	+	-	Escherichia coli				+										R	S	R	S	S	S	S	S	S	S	R	S	S				
951	18131	30	G2P1L1	III	clear	-	-	-	-	NG																																	
952	26715	23	Primi	II	clear	-	-	-	-	NG																																	
953	29062	28	G2P1L1	I	clear	-	-	-	-	NG																																	
954	4015	21	Primi	III	clear	-	-	-	-	NG																																	
955	4329	25	Primi	I	turbid	+	-	+	+	Escherichia coli	+	+												S	S	S	S	S	S	S	S	R	S	S	S	S				
956	27792	23	Primi	III	turbid	-	-	-	-	NG																																	
957	880	27	G2P1L1	II	clear	-	-	-	-	NG																																	
958	3531	25	Primi	II	turbid	-	-	-	-	NG																																	

959	4182	25	Primi	I	clear	-	-	-	-	NG																																	
960	4592	22	Primi	I	clear	-	-	-	-	NG																																	
961	27597	25	G2P1L1	III	turbid	-	-	-	-	NG																																	
962	4375	20	Primi	II	clear	-	-	-	-	NG																																	
963	3228	35	G2P1L1	I	clear	-	-	-	-	NG																																	
964	4506	30	G2P1L1	III	clear	-	-	-	-	NG																																	
965	9953	25	Primi	II	clear	-	-	-	-	NG																																	
966	5318	35	G3P2L2	III	clear	-	-	-	-	NG																																	
967	3408	20	G2P1L1	III	clear	-	-	-	-	NG																																	
968	4217	19	Primi	I	clear	-	-	-	-	NG																																	
969	2235	26	G3P1L1A1	III	clear	-	-	-	-	NG																																	
970	46342	28	G3P1L1A1	III	clear	-	-	-	-	NG																																	
971	12633	21	G2P1L1	I	clear	-	-	-	-	NG																																	
972	2216	25	G3P2L2	II	clear	-	-	-	-	NG																																	
973	4531	23	G2P1L1	III	turbid	-	-	-	-	NG																																	
974	2097	38	G3P1L1A1	II	turbid	-	-	-	-	NG																																	
975	2345	26	G3P0L0A2	II	clear	-	-	-	-	NG																																	
976	2315	24	G2P1L1	III	clear	-	-	-	-	NG																																	
977	3214	21	Primi	I	clear	-	-	-	-	NG																																	
978	4326	22	G2P1L1	I	clear	-	-	-	-	NG																																	
979	2186	35	G4P2L2A1	II	clear	-	-	-	-	NG																																	
980	4357	28	G2P1L1	III	clear	-	-	-	-	NG																																	
981	2138	27	G3P2L2	II	clear	-	-	-	-	NG																																	
982	1263	32	G3P2L2	II	clear	-	-	-	-	NG																																	
983	3476	25	G2P1L1	I	clear	-	-	-	-	NG																																	
984	7653	26	G3P1L1A1	I	clear	-	-	-	-	NG																																	
985	2367	24	G2P1L1	II	clear	-	-	-	-	NG																																	
986	4651	20	Primi	I	clear	-	-	-	-	NG																																	
987	4653	29	G3P1L1A1	III	clear	-	-	-	-	NG																																	
988	2764	29	G4P1L1A2	II	clear	-	-	-	+	NG																																	
989	3289	24	G3P0L0A2	I	clear	-	-	-	-	NG																																	

990	5632	24	G2P0L0A1	II	clear	-	-	-	-	NG																																			
991	1240	30	G4P1L1A2	III	clear	-	-	-	-	NG																																			
992	2387	35	G3P2L2	II	clear	-	-	-	-	NG																																			
993	3451	25	G2P0L0A1	II	turbid	-	-	-	-	NG																																			
994	3570	28	G3P1L1A1	III	clear	-	-	-	-	NG																																			
995	5243	24	G3P0L0A2	II	turbid	-	-	-	-	NG																																			
996	1778	26	G3P2L2	II	turbid	-	-	-	+	NG																																			
997	5631	23	G3P1L1A1	I	clear	-	-	-	-	NG																																			
998	3475	20	Primi	I	clear	-	-	-	-	NG																																			
999	4520	20	G2P1L1	I	clear	-	-	-	+	NG																																			
1000	3216	25	G2 P1L1	I	clear	-	-	-	-	NG																																			